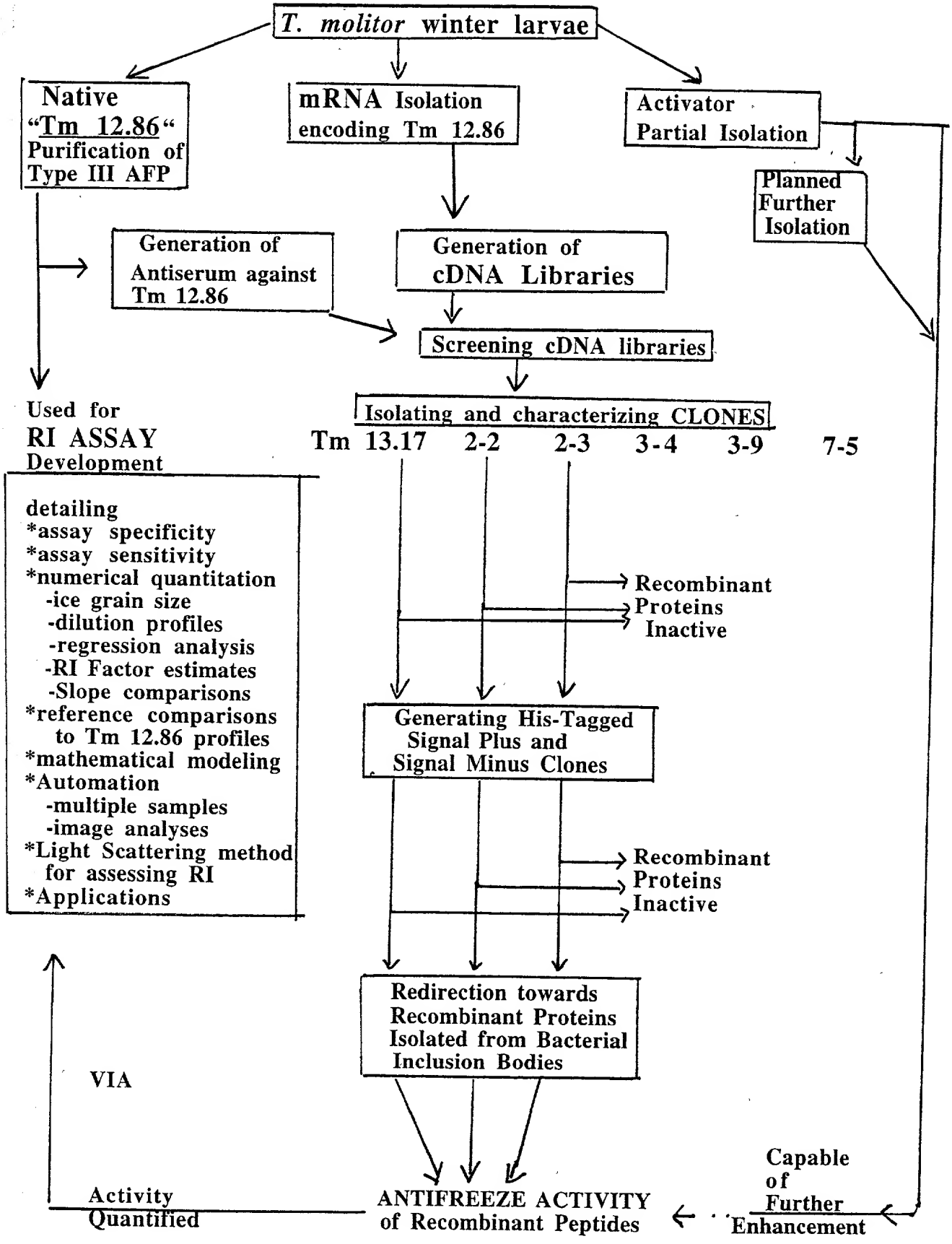
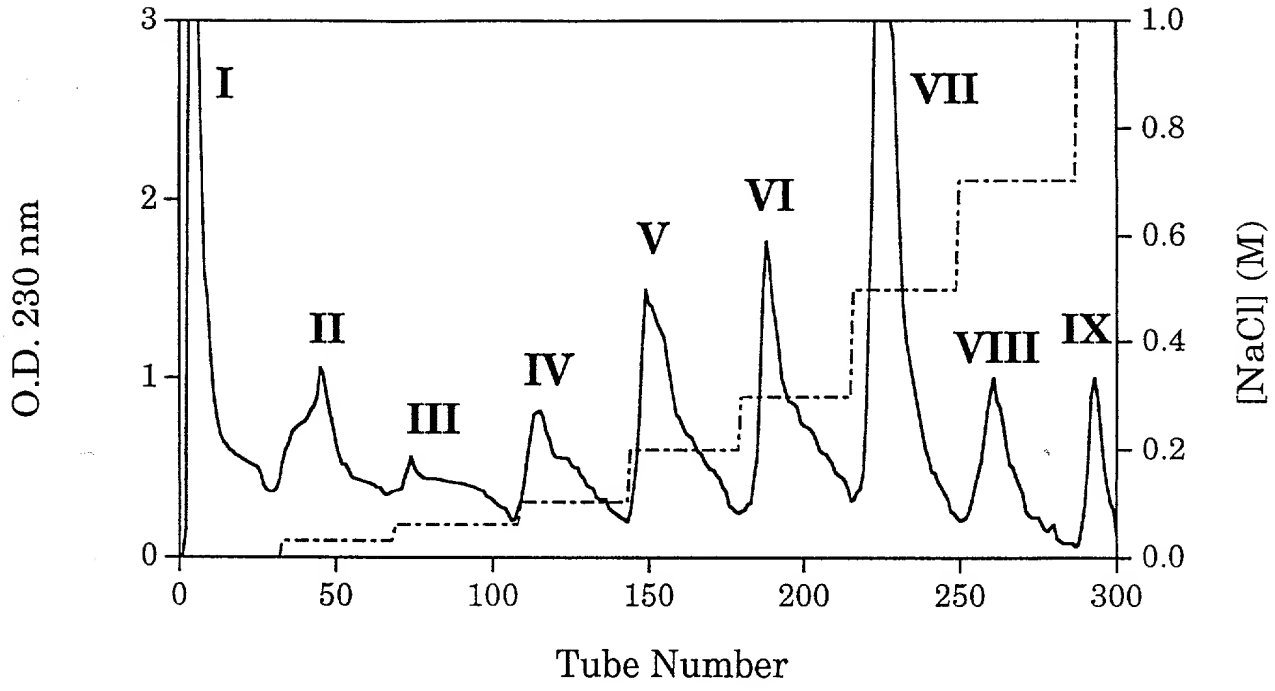

T. molitor winter larvae
Native "Tm 12.86" Purification of Type III AFP
mRNA Isolation encoding Tm 12.86
Activator Partial Isolation
Planned Further Isolation
Generation of Antiserum against Tm 12.86
Generation of cDNA Libraries
Screening cDNA libraries
Isolating and characterizing CLONES
Tm 13.17
2-2
2-3
3-4
3-9
7-5
Used for RI ASSAY Development
detailing
*assay specificity
*assay sensitivity
*numerical quantitation
-ice grain size
-dilution profiles
-regression analysis
-RI Factor estimates
-Slope comparisons
*reference comparisons to Tm 12.86 profiles
*mathematical modeling
*Automation
-multiple samples
-image analyses
*Light Scattering method for assessing RI
*Applications
Recombinant Proteins Inactive
Generating His-Tagged Signal Plus and Signal Minus Clones
Recombinant Proteins Inactive
Redirection towards Recombinant Proteins Isolated from Bacterial Inclusion Bodies
VIA
Activity Quantified
ANTIFREEZE ACTIVITY of Recombinant Peptides
Capable of Further Enhancement

FIG 1.0

A.


O.D. 230 nm
[NaCl] (M)
Tube Number
I
II
III
IV
V
VI
VII
VIII
IX
0
1
2
3
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
300

B.

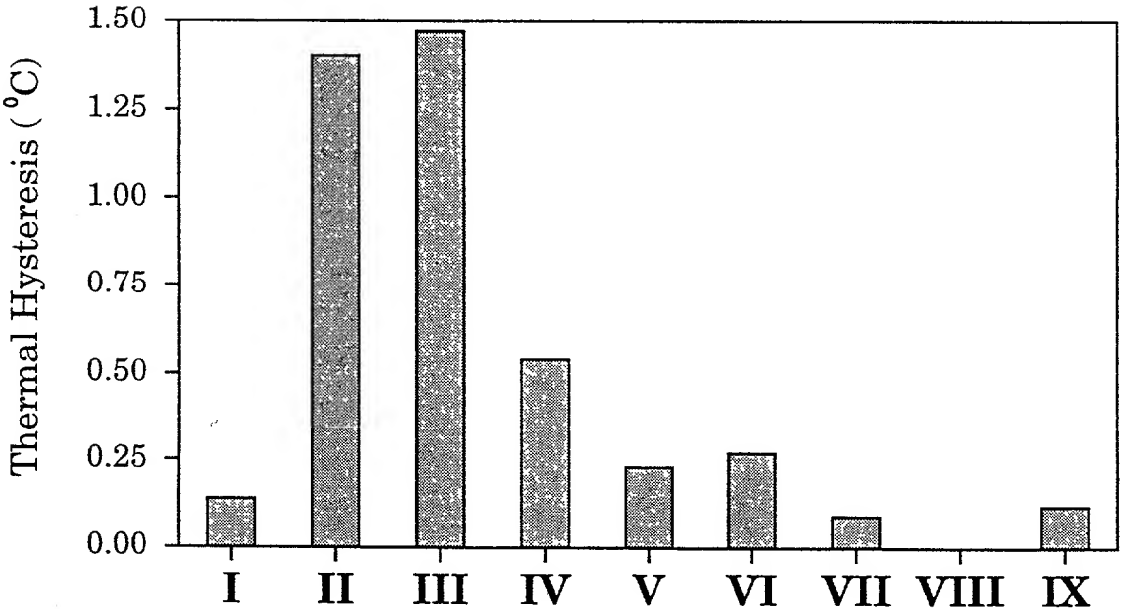

Thermal Hysteresis (°C)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
I
II
III
IV
V
VI
VII
VIII
IX
Ion Exchange Peak at 50 mg/ml

FIG 1.1

TO20090" 96292860

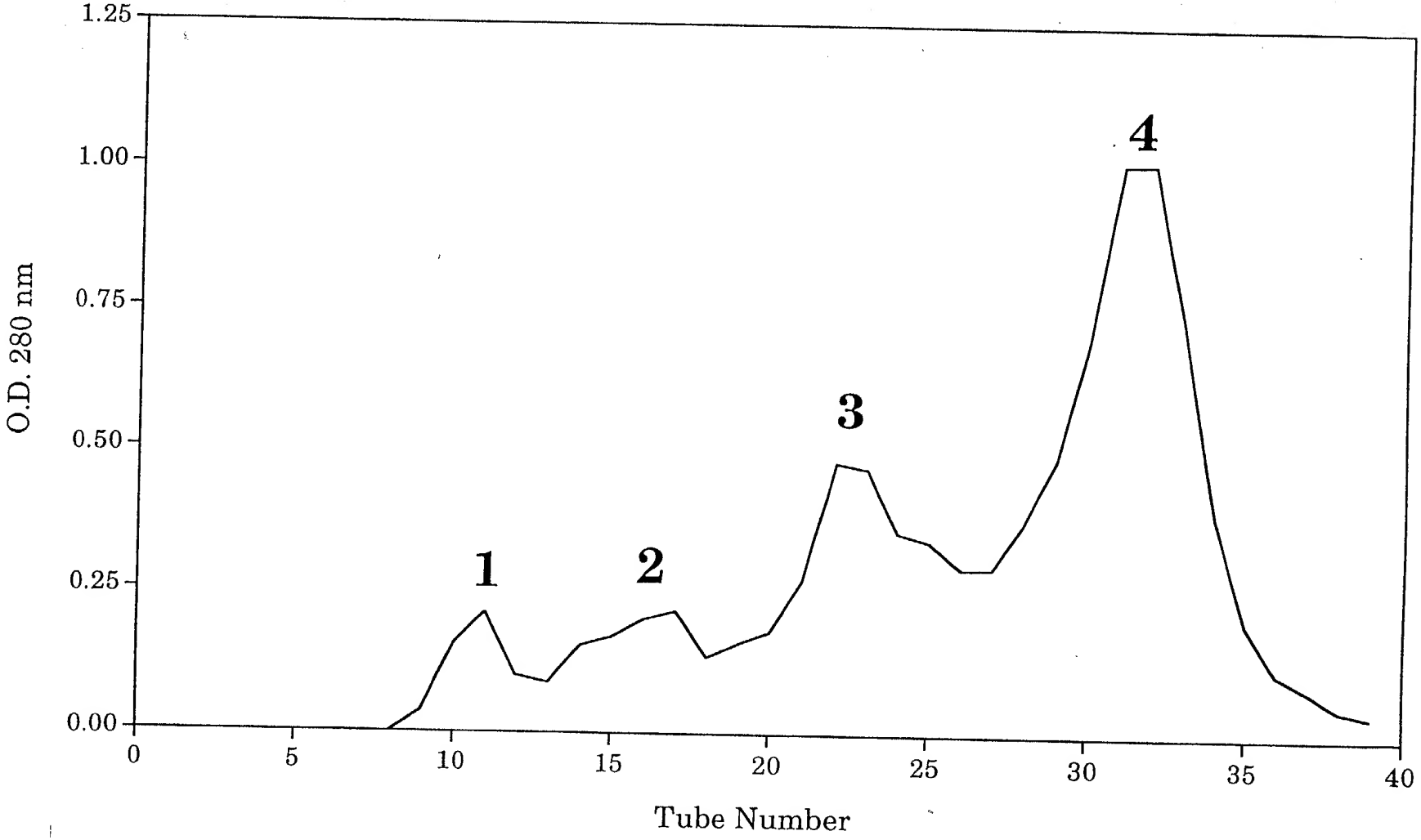
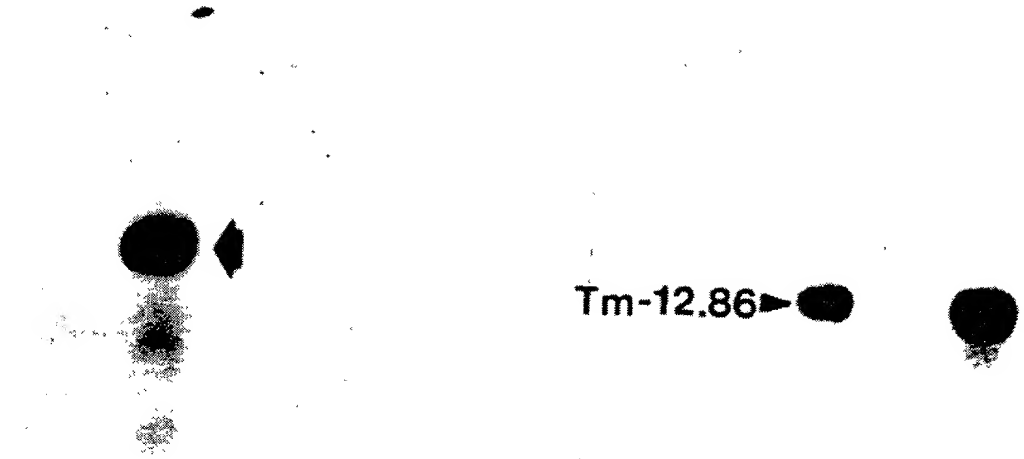


FIG 1.2



Patent - 05/06/2008

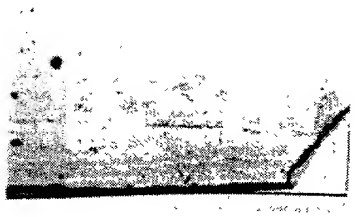


FIG 1.3

12.5 25

FIG 1.4

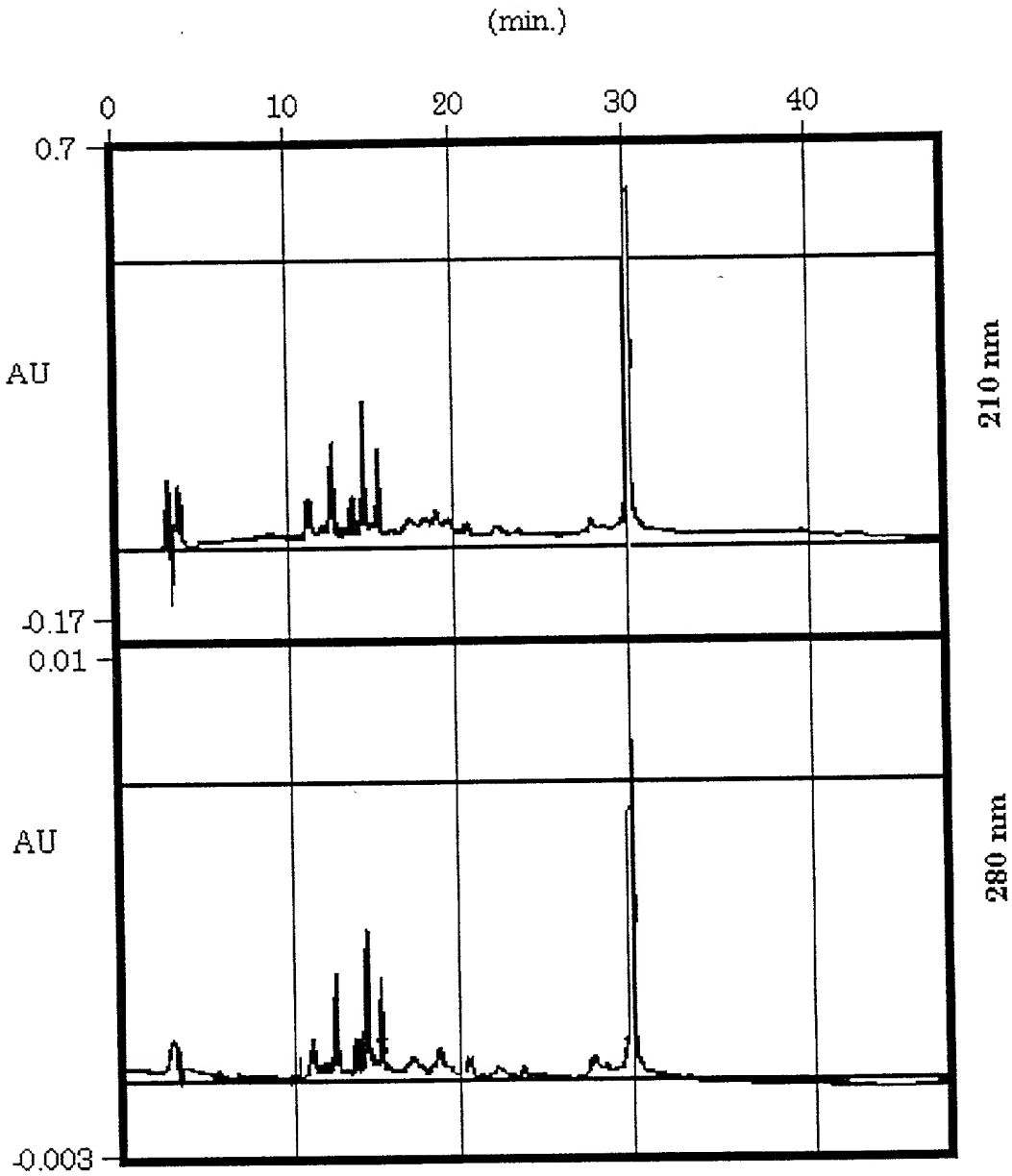


FIG 1.5

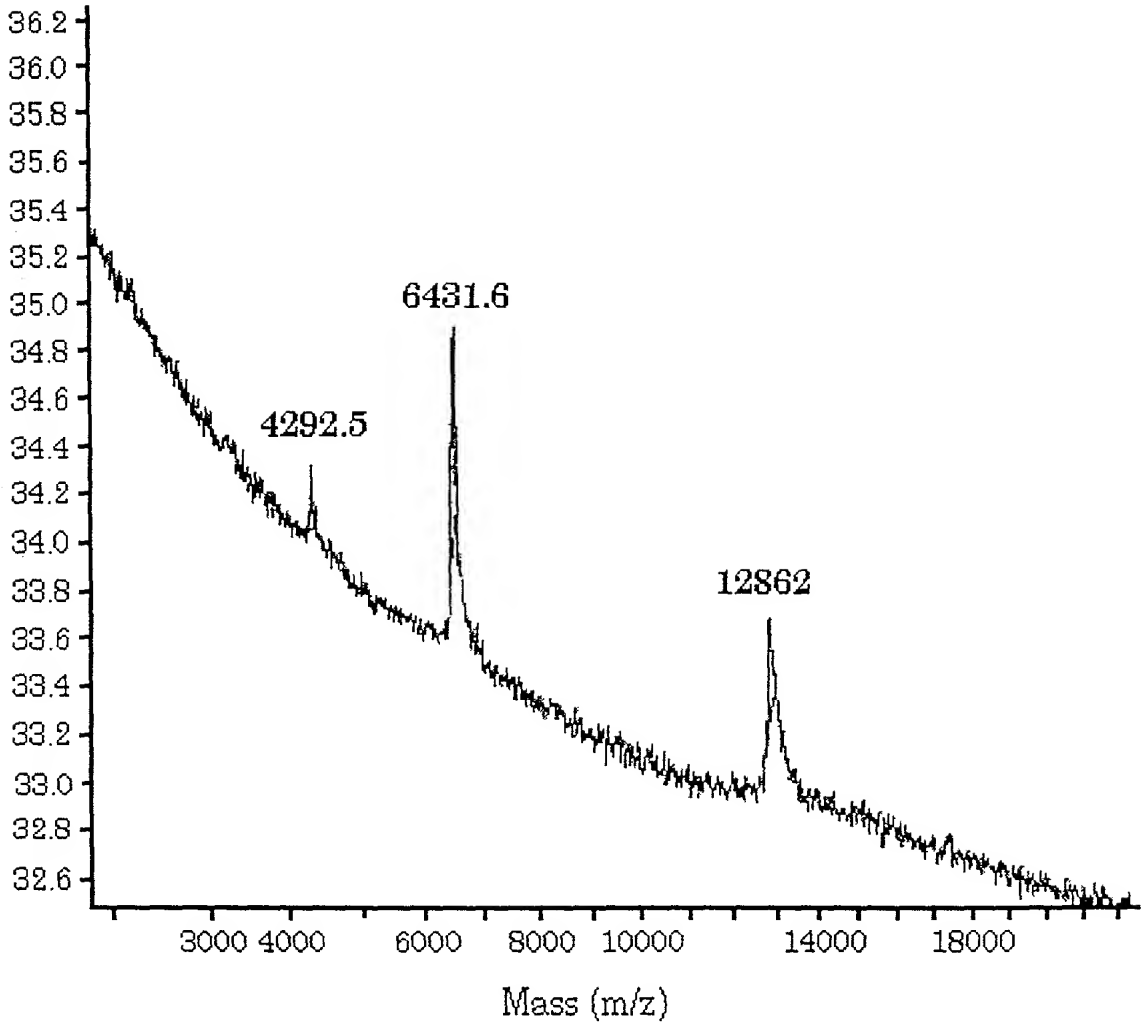

36.2
36.0
35.8
35.6
35.4
35.2
35.0
34.8
34.6
34.4
34.2
34.0
33.8
33.6
33.4
33.2
33.0
32.8
32.6
6431.6
4292.5
12862
3000 4000
6000
8000
10000
14000
18000
Mass (m/z)

FIG 1.6

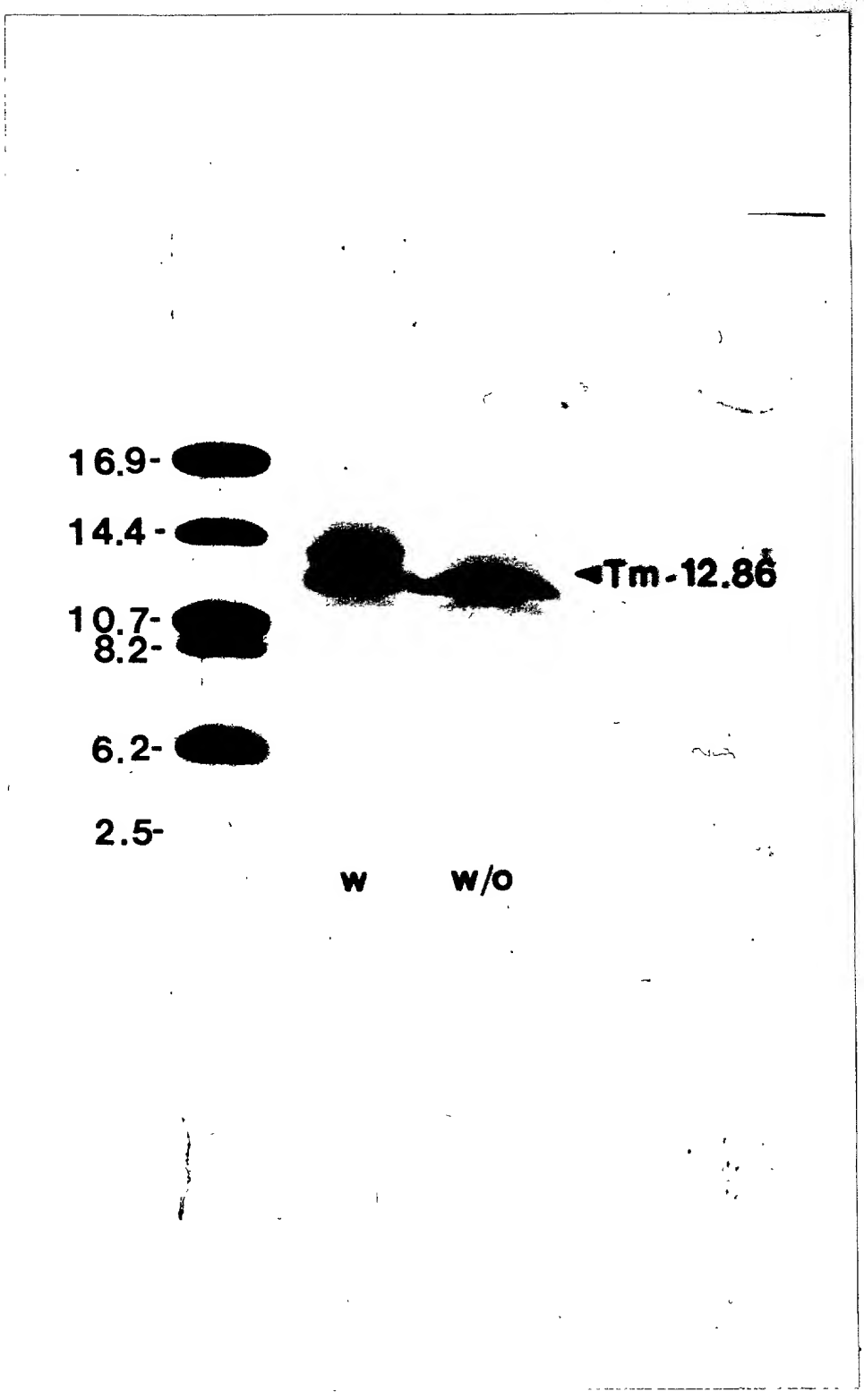


FIG 1.7

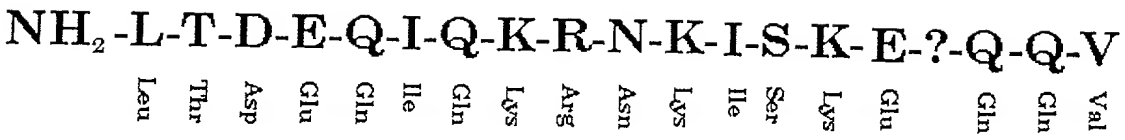

NH2-L-T-D-E-Q-I-Q-K-R-N-K-I-S-K-E-?-Q-Q-V
Leu
Thr
Asp
Glu
Gln
Ile
Gln
Lys
Arg
Asn
Lys
Ile
Ser
Lys
Glu
Gln
Gln
Val

FIG. 1.8

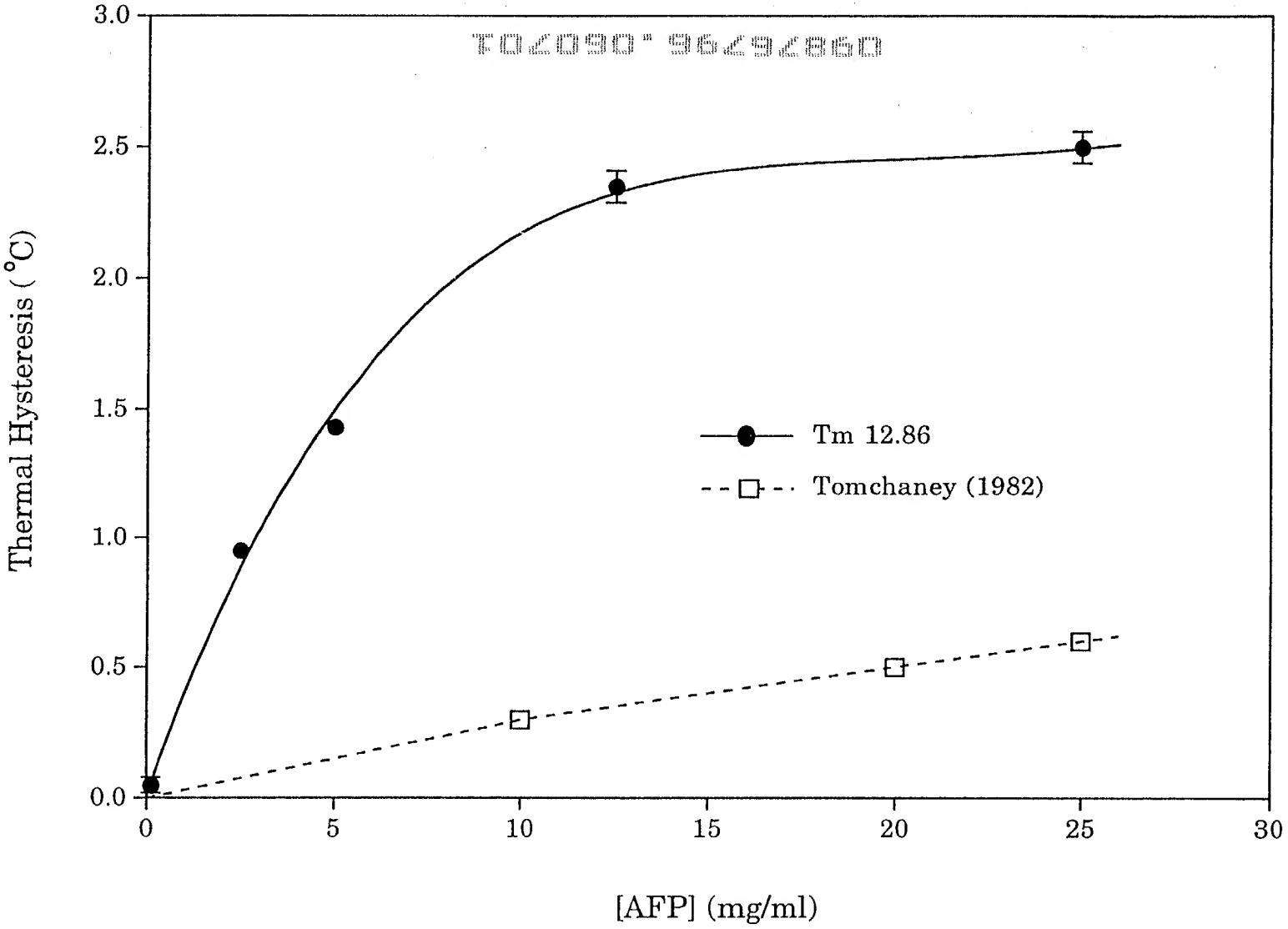

Thermal Hysteresis (°C)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
[AFP] (mg/ml)
Tm 12.86
Tomchaney (1982)

FIG 1.9

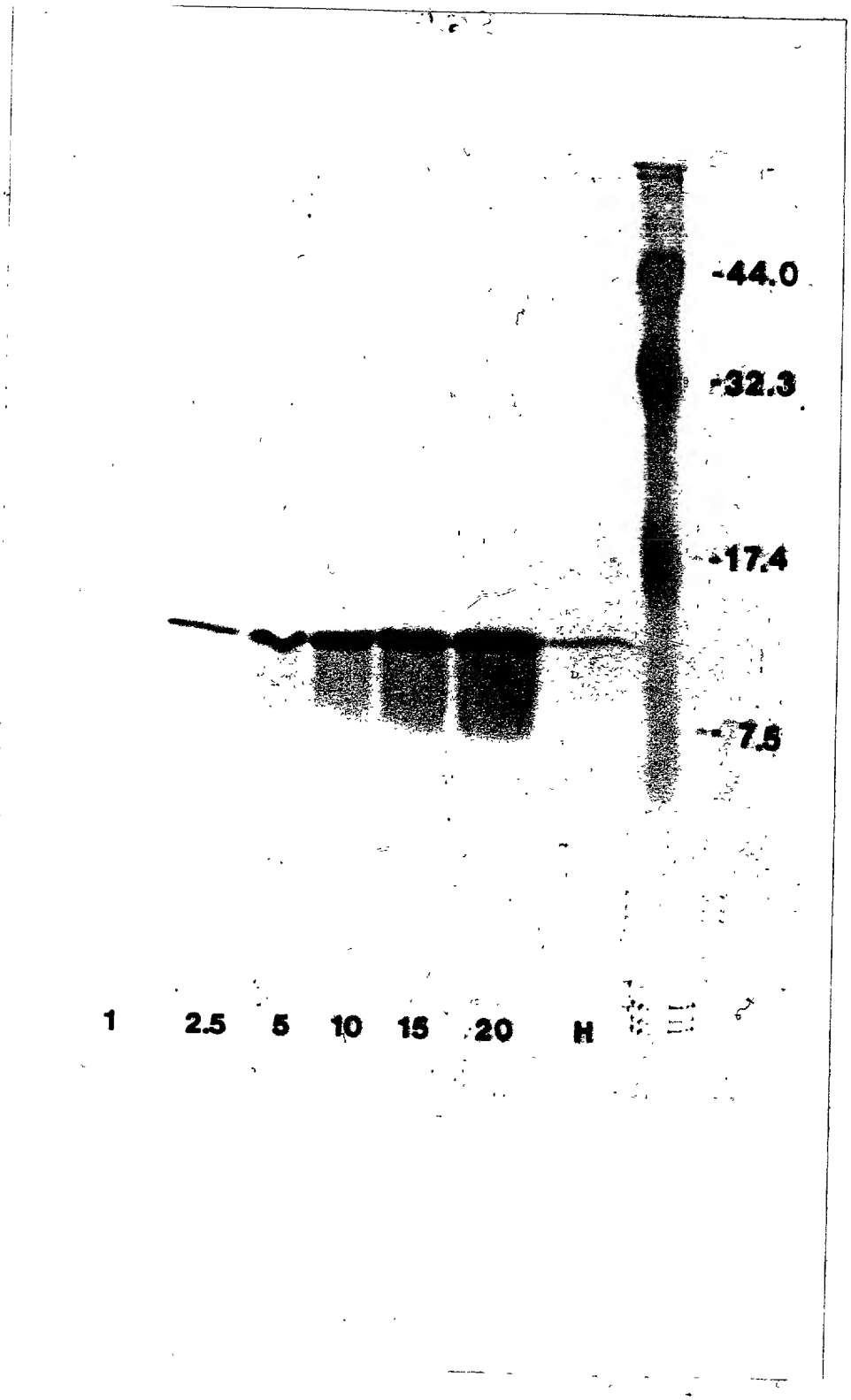


FIG 1.10

TO2090 96292860

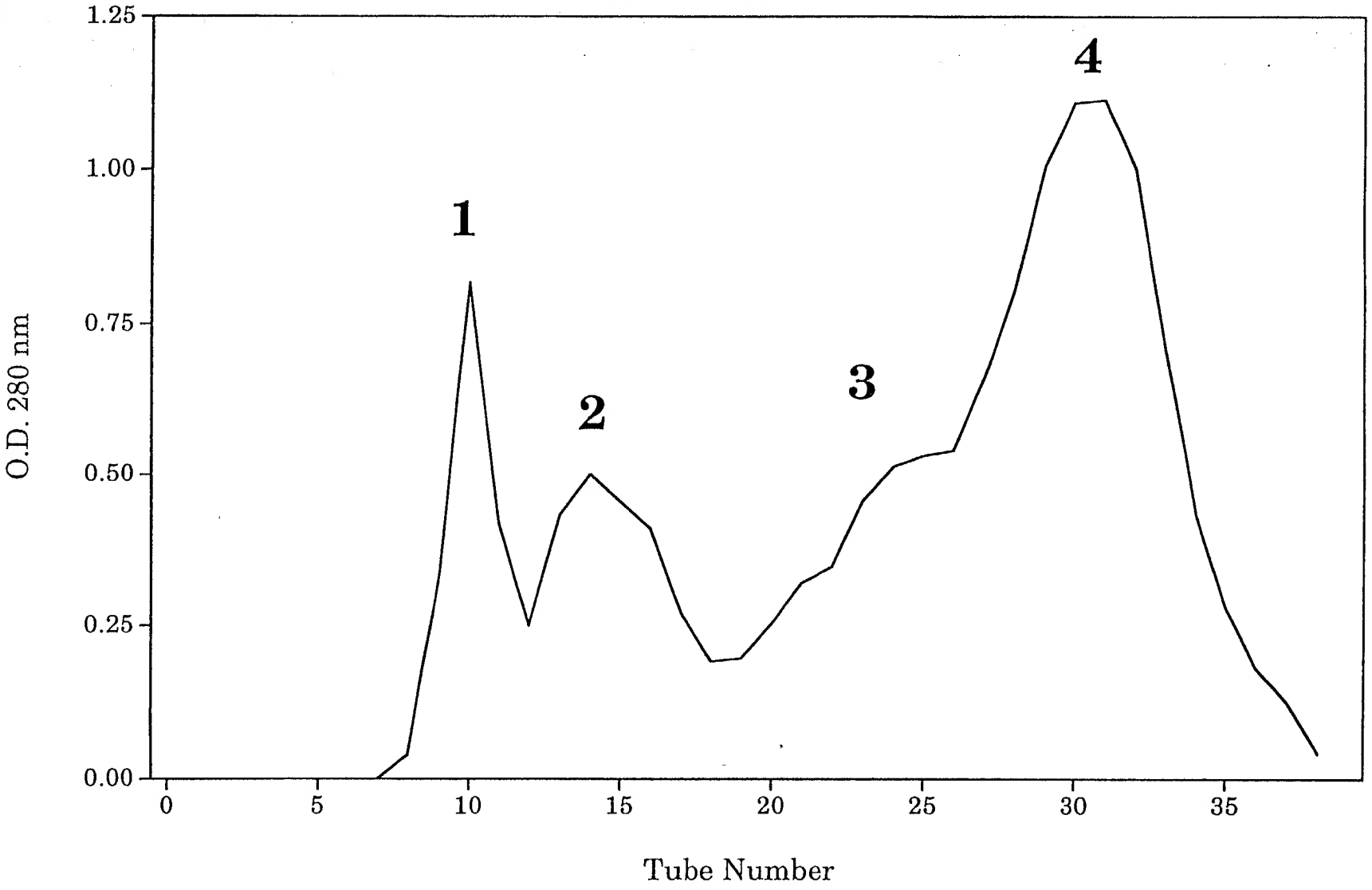


FIG 1.11

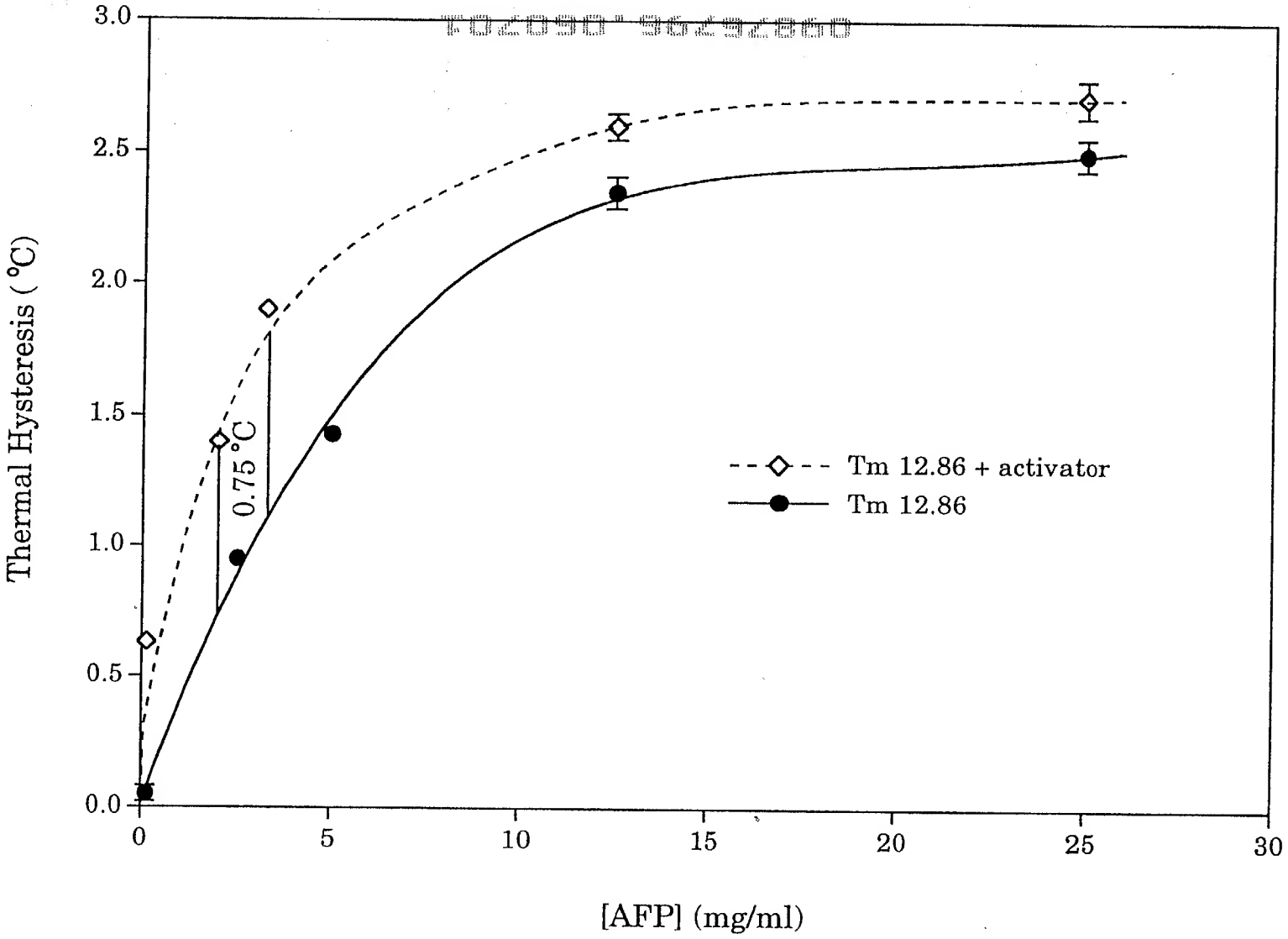

Thermal Hysteresis (°C)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
[AFP] (mg/ml)
0.75 °C
Tm 12.86 + activator
Tm 12.86

FIG 1.12

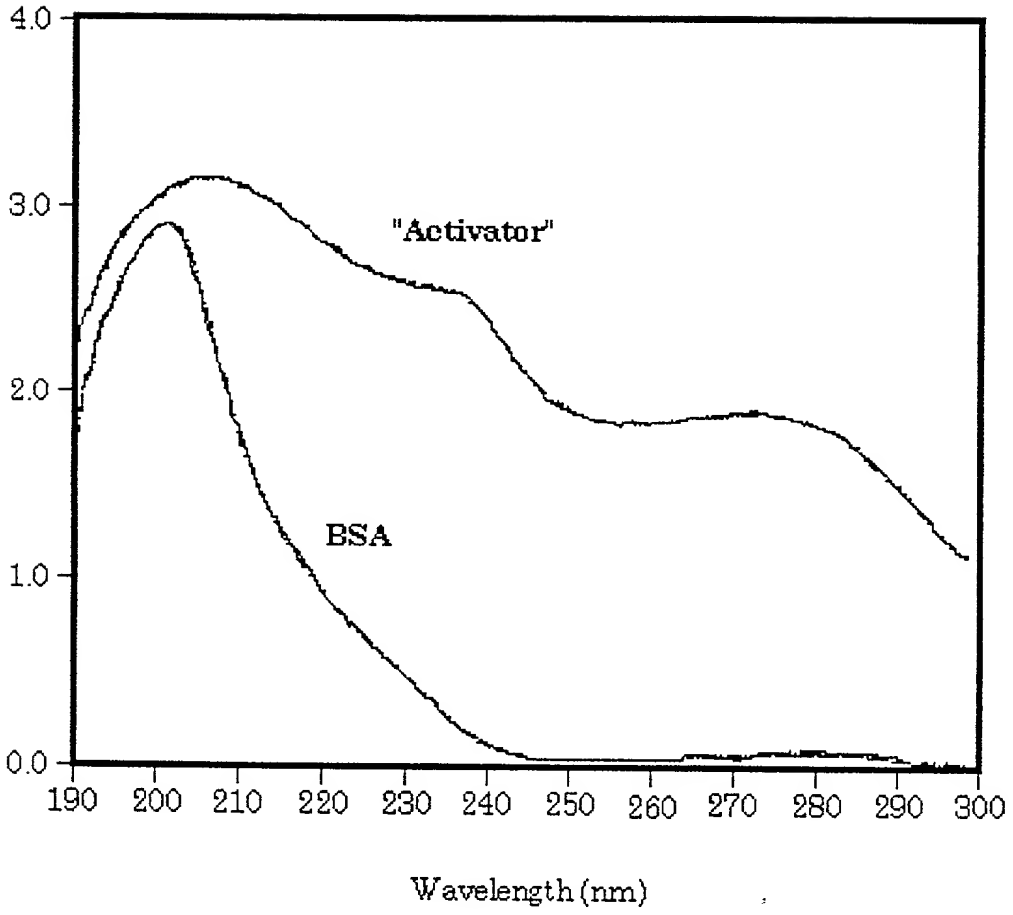

4.0
3.0
2.0
1.0
0.0
"Activator"
BSA
190
200
210
220
230
240
250
260
270
280
290
300
Absorbance
Wavelength (nm)

FIG 1.13

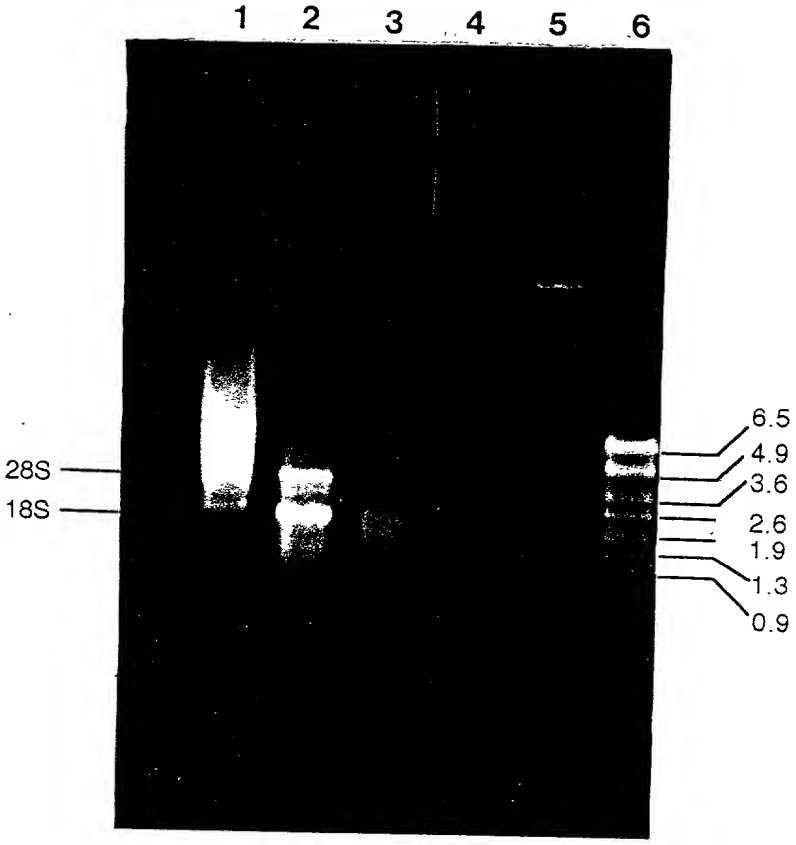


FIG 2.0

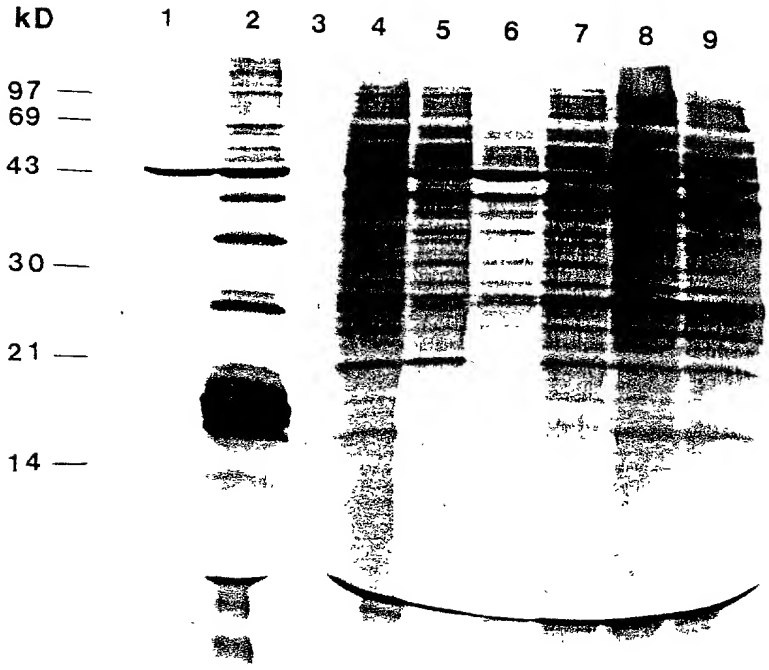


FIG 2.1

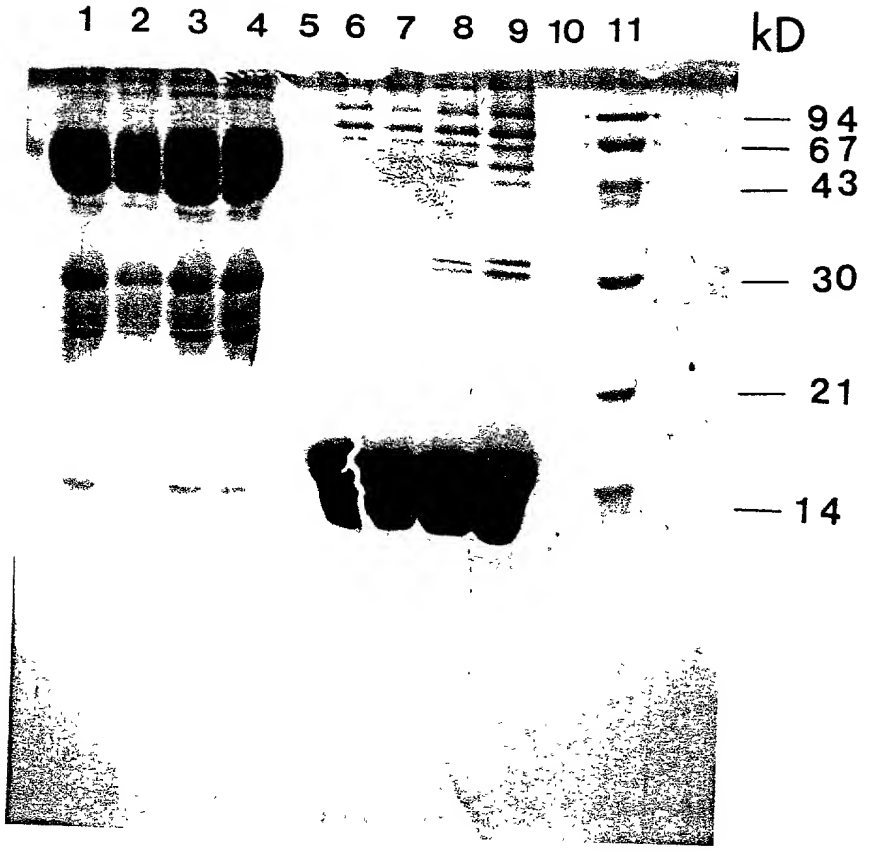


FIG 2.2

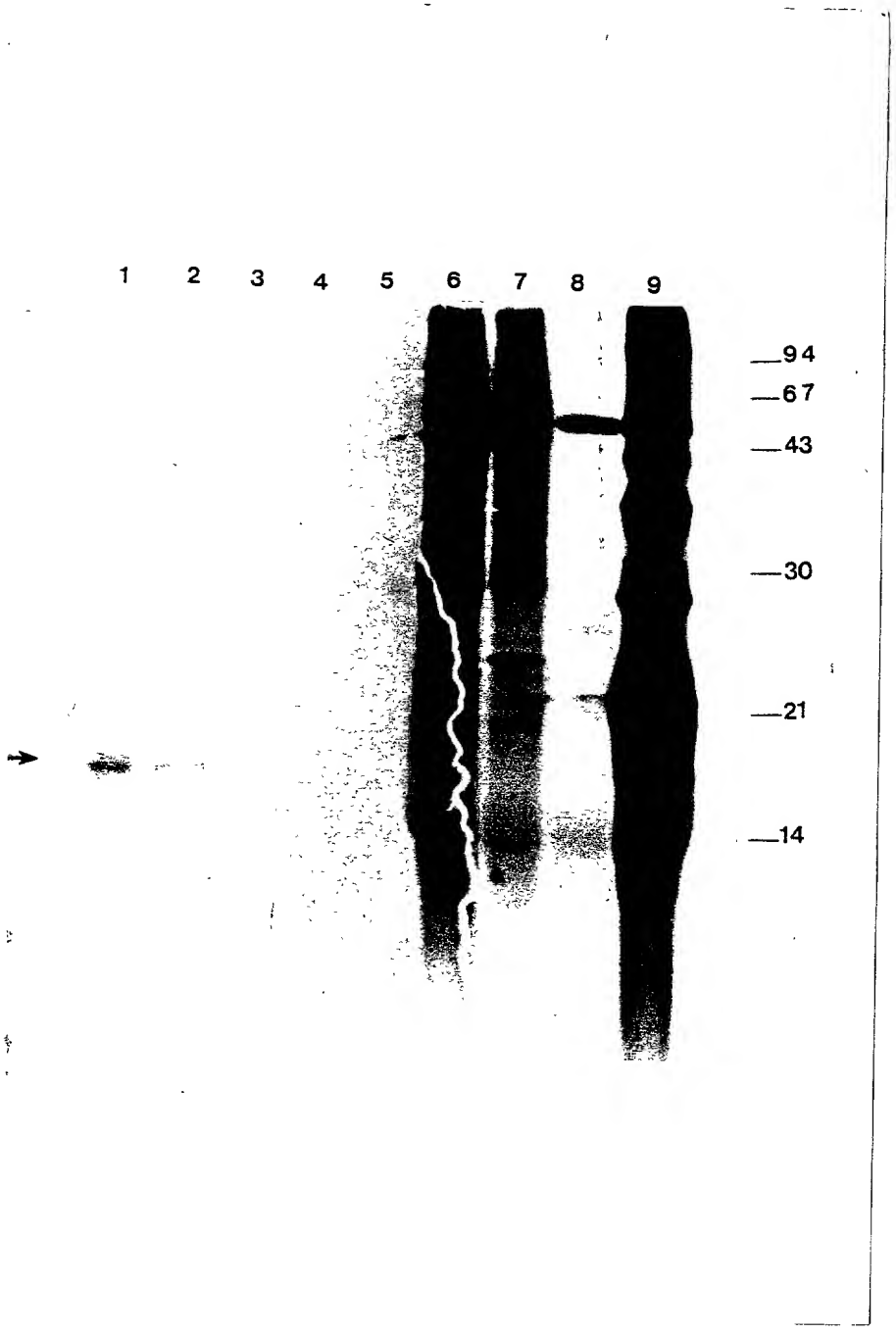


FIG 2.3

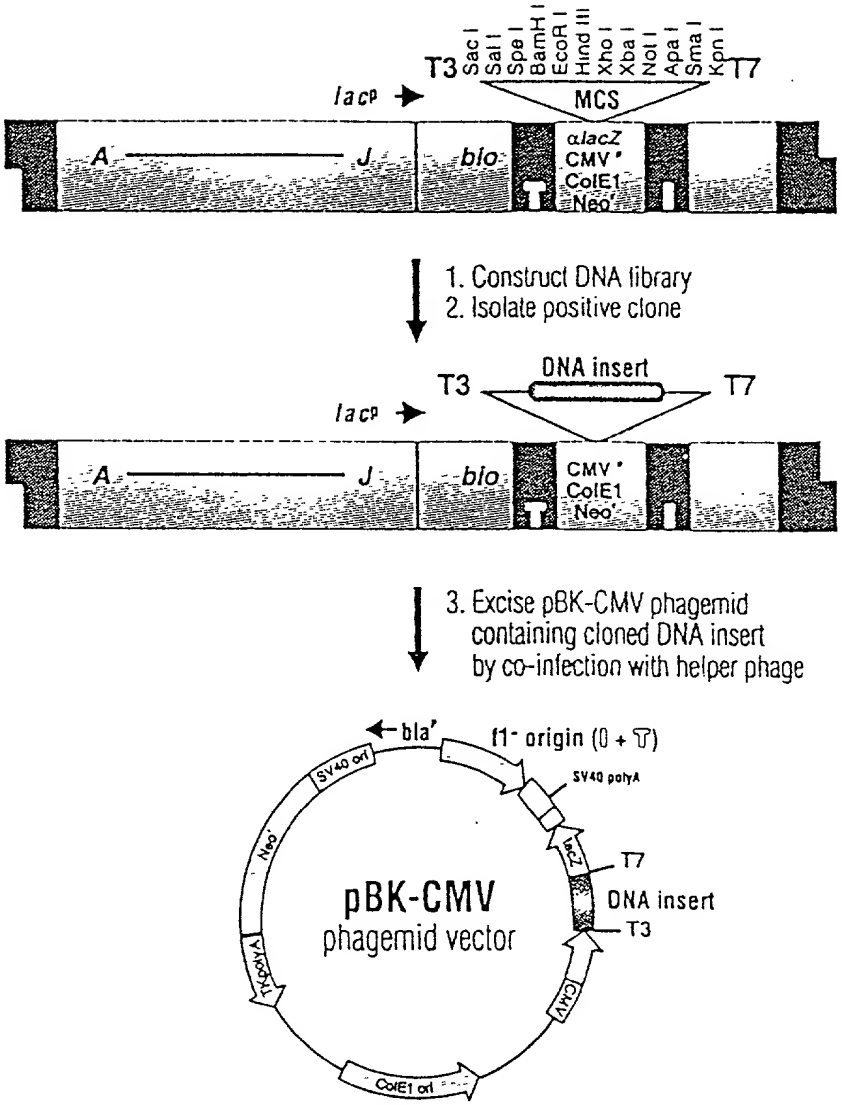

T3
Sac I
Sal I
Spe I
BamH I
EcoR I
Hind III
Xho I
Xba I
Not I
Apa I
Sma I
Kpn I
T7
MCS
lacP
A
J
bio
αlacZ
CMV'
ColE1
Neo'
1. Construct DNA library
2. Isolate positive clone
DNA insert
3. Excise pBK-CMV phagemid
containing cloned DNA insert
by co-infection with helper phage
bla'
f1- origin (0 + 7)
SV40 polyA
lacZ
pBK-CMV
phagemid vector
SV40 ori
Neo'
CMV
ColE1 ori

FIG. 2.4 a

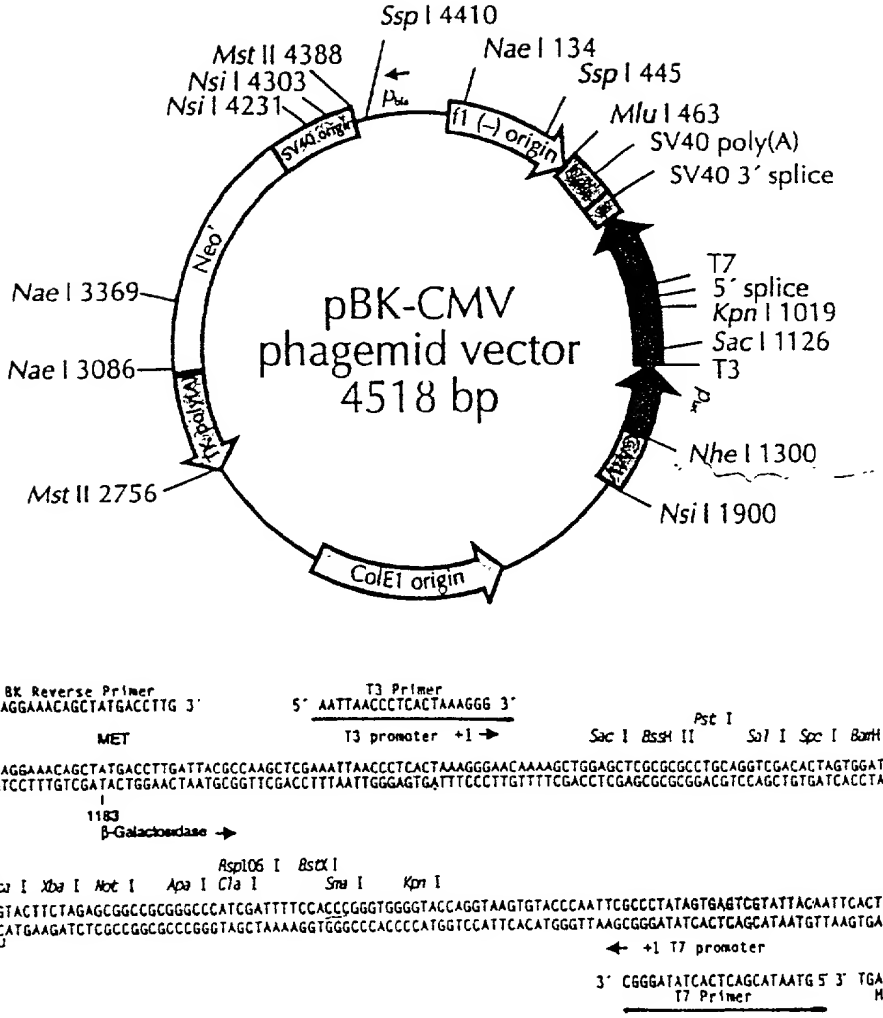

Ssp I 4410
Nae I 134
Mst II 4388
Nsi I 4303
Nsi I 4231
Ssp I 445
Mlu I 463
SV40 poly(A)
SV40 3´ splice
T7
5´ splice
Kpn I 1019
Sac I 1126
T3
Nae I 3369
Nae I 3086
pBK-CMV
phagemid vector
4518 bp
Nhe I 1300
Mst II 2756
Nsi I 1900
ColE1 origin
f1 (-) origin
Neo
BK Reverse Primer
5´ ACAGGAAACAGCTATGACCTTG 3´
T3 Primer
5´ AATTAACCCTCACTAAAGGG 3´
1200
MET
T3 promoter +1
Pst I
Sac I BssH II
Sal I Spc I BamH I
EcoR I
5´ TCACACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCGCGCGCCTGCAGGTCGACACTAGTGGATCCAAAG
3´ AGTGTGTCCTTTGTCGATACTGGAACTAATGCGGTTCGACCTTTAATTGGGAGTGATTTCCCTTGTTTTCGACCTCGAGCGCGCGGACGTCCAGCTGTGATCACCTAGGTTTCTTAA
1183
β-Galactosidase
Xho I
Hind III
Asp106 I
BstX I
Sca I Xba I Not I Apa I Cla I
Sma I
Kpn I
AATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCGCGGGCCCATCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACA 3´ (+)
GTTTTTCGAAGAGCTCTCATGAAGATCTCGCCGGCGCCCGGGTAGCTAAAAGGTGGGCCCACCCCATGGTCCATTCACATGGGTTAAGCGGGATATCACTCAGCATAATGTTAAGTGACCGGCAGCAAAATGT 5´ (-)
+1 T7 promoter
951
3´ CGGGATATCACTCAGCATAATG 5´ 3´ TGACCGGCAGCAAAATG 5´
T7 Primer
M13-20 Primer

FIG. 2.4 b

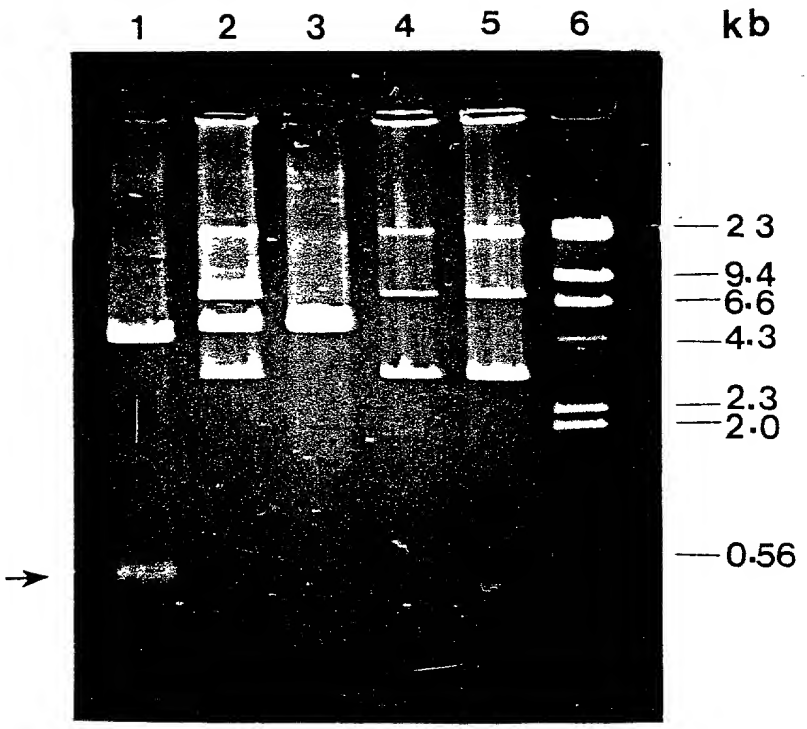


FIG 2.5

DNA sequence of Tm 13.17 cDNA clone

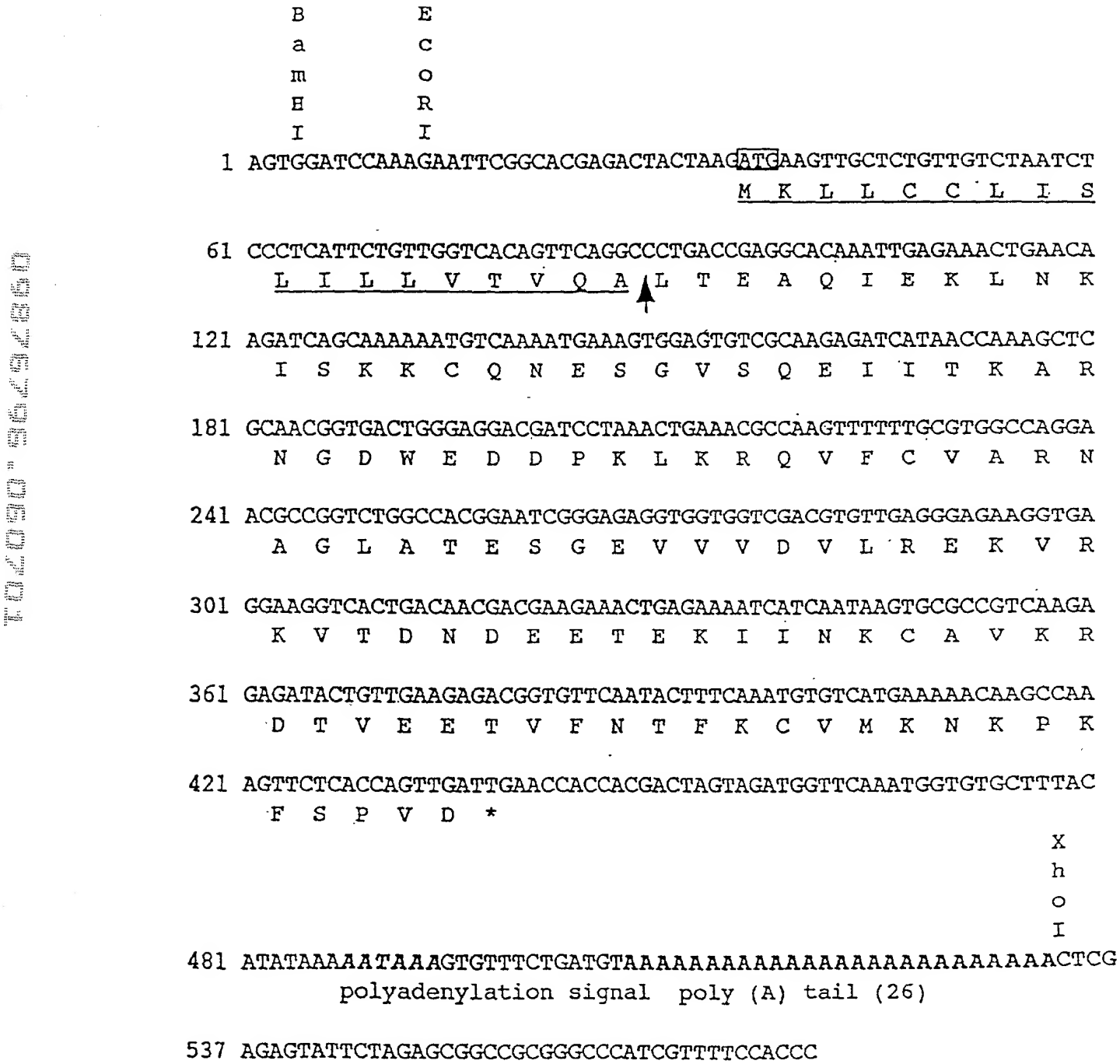


FIG 2.6a

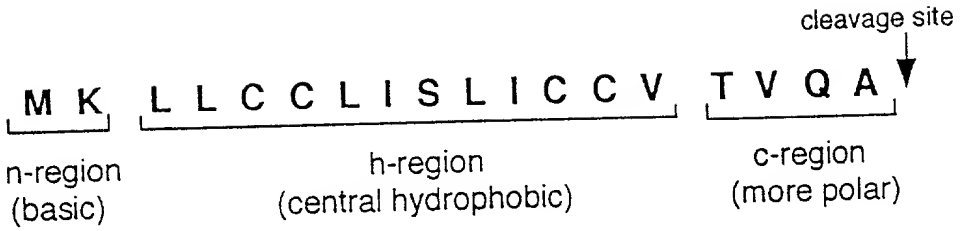

cleavage site
M K
L L C C L I S L I C C V
T V Q A
n-region
(basic)
h-region
(central hydrophobic)
c-region
(more polar)

FIG 2.6b

A. Mature Tm 13.17 amino acid residure

```
  1  LTEAQIEKLN KISKKCQNES GVSQEIITKA RNGDWEDDPK LKRQVFCVAR

 51  NAGLATESGE VVVDVLREKV RKVTDNDEET EKIINKCAVK RDTVEETVFN

101  TFKCVMKNKP KFSPVD
```

B. Summary of the composition analysis for the mature Tm 13.17 sequence:

Residue	Number	Mole Percent
A = Ala	6	5.172
B = Asx	0	0.000
C = Cys	4	3.448
D = Asp	8	6.897
E = Glu	**13**	**11.207**
F = Phe	4	3.448
G = Gly	4	3.448
H = His	0	0.000
I = Ile	6	5.172
K =Lys	**16**	**13.793**
L = Leu	5	4.310
M = Met	1	0.862
N = Asn	8	6.897
P = Pro	3	2.586
Q = Gln	4	3.448
R = Arg	6	5.172
S = Ser	5	4.310
T = Thr	8	6.897
V =Val	**14**	**12.069**
W = Trp	1	0.862
Y = Tyr	0	0.000
Z = Glx	0	0.000

Molecular weight = 13171.96; Residues = 116; Average Residue Weight = 113.551

Charge = 1; Isoelectric point = 7.74.

FIG 2.6c

PCT/US99/30876

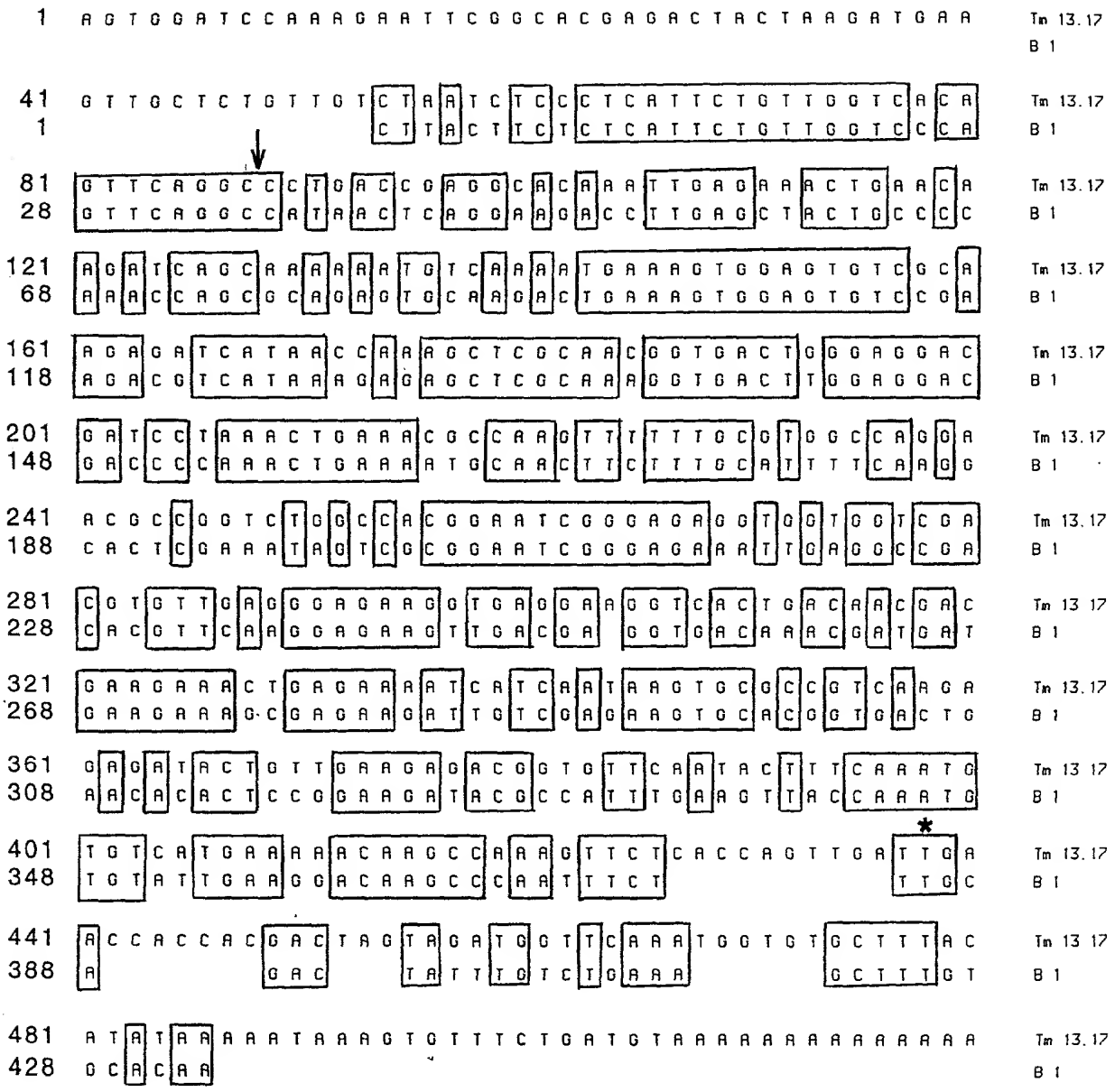

1 AGTGGATCCAAAGAATTCGGCACGAGACTACTAAGATGAA Tm 13.17 B 1
41 GTTGCTCTGTTGTCTAATCTCCCTCATTCTGTTGGTCACA Tm 13.17 B 1
1 CTTACTTCTCATTCTGTTGGTCCA
81 GTTCAGGCCCTGACCGAGGCAACAAATTGAGAAACTGAACA Tm 13.17 B 1
28 GTTCAGGCCARTAACTCAGGAAGACCTTGAGCTACTGCCCC
121 AGATCAGCAAAAAATGTCAAAATGAAAAGTGGAGTGTCGCA Tm 13.17 B 1
68 AAACCAGCGCAGAGTGCAAGACTGAAAAGTGGAGTGTCCGA
161 AGAGATCATAACCCAAAGCTCGCAACGGTGACTGGGAGGAC Tm 13.17 B 1
118 AGACGTCATAAAGAGAGCTCGCAAAGGTGACTTGGAGGAC
201 GATCCTAAACTGAAAACGCCAAGTTTTTGCGTGGCCAGGA Tm 13.17 B 1
148 GACCCCAAACTGAAAATGCAACCTTCTTTGCATTTTCAAGG
241 ACGCCGGTCTGGCCACGGAATCGGGAGAGGGTGGTGGTCGA Tm 13.17 B 1
188 CACTCGAAATAGTCGCGGAATCGGGAGAAATTGAGGCCGA
281 CGTGTTGAGGGAGAAGGTGAGGAAGGTCACTGACAACGAC Tm 13 17 B 1
228 CACGTTCAAGGAGAAGTTGACGA GGTGACAAACGATGAT
321 GAAGAAACTGAGAAAATCATCAATAAGTGCGCCGTCAAGA Tm 13.17 B 1
268 GAAGAAAGCGAGAAGATTGTCGAGAAGTGCACGGTGACTG
361 GAGATACTGTTGAAGAGACGGTGTTCAATACTTTCAAATG Tm 13 17 B 1
308 AACACACTCCGGAAGATACGCCATTTGAAGTTACCAAATG
401 TGTCATGAAAAACAAGCCAAAGTTCTCACCAGTTGATTGA Tm 13.17 B 1
348 TGTATTGAAGGACAAGCCCAATTCT TTGC
441 ACCACCACGACTAGTAGATGGTTCAAATGGTGTGCTTTAC Tm 13 17 B 1
388 A GAC TATTTGTCTGAAA GCTTTGT
481 ATATAAAATAAAGTGTTTCTGATGTAAAAAAAAAAAAAAA Tm 13.17 B 1
428 GCACAA

FIG 2.7

```
Tm 13.17      3  EAQIEKLNKISKKCQNESGVSQEIITKARNGDWEDDPKLKRQVFCVARNA 52
                 |.. |||.. |. |..|||||:| :.|.|| :  |||||| :.||: :.|
AFP-3         1  ETPREKLKQHSDACKAESGVSEESLNKVRNREEVDDPKLKEHAFCILKRA 50

Tm 13.17     53  GLATESGEVVVDVLREKVRKVTDNDEETEKIINKCAVKRDTVEETVFNTF 102
                 |: ..|||. :| ::.|.:. .::.|..:.::.|||||:||.:..  : |
AFP-3        51  GFIDASGEFQLDHIKTKFKENSEHPEKVDDLVAKCAVKKDTPQHSSADFF 100

Tm 13.17    103  KCVMKNKP 110
                 ||| .|:.
AFP-3       101  KCVHDNRS 108
```

Percent identity: 39.8 (identical amino acids; Percent similarity: 58.3 (identical amino acids plus conservative amino acids).

FIG 2.8

Document 2 - 05/05/2005

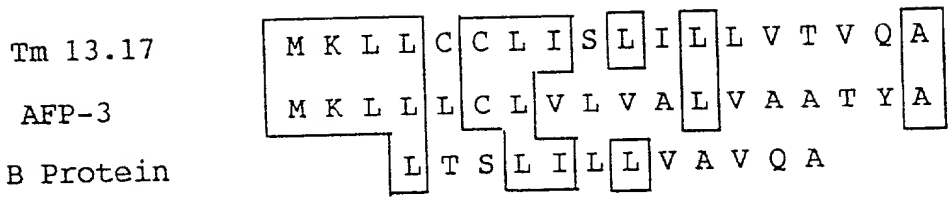


FIG 2.9

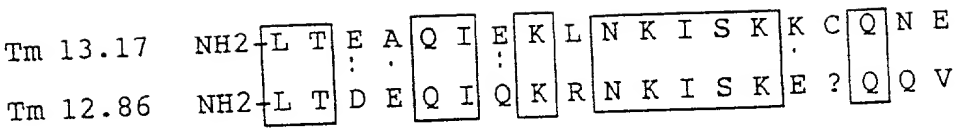

Tm 13.17 NH2 L T E A Q I E K L N K I S K K C Q N E
Tm 12.86 NH2 L T D E Q I Q K R N K I S K E ? Q Q V

FIG 2.10

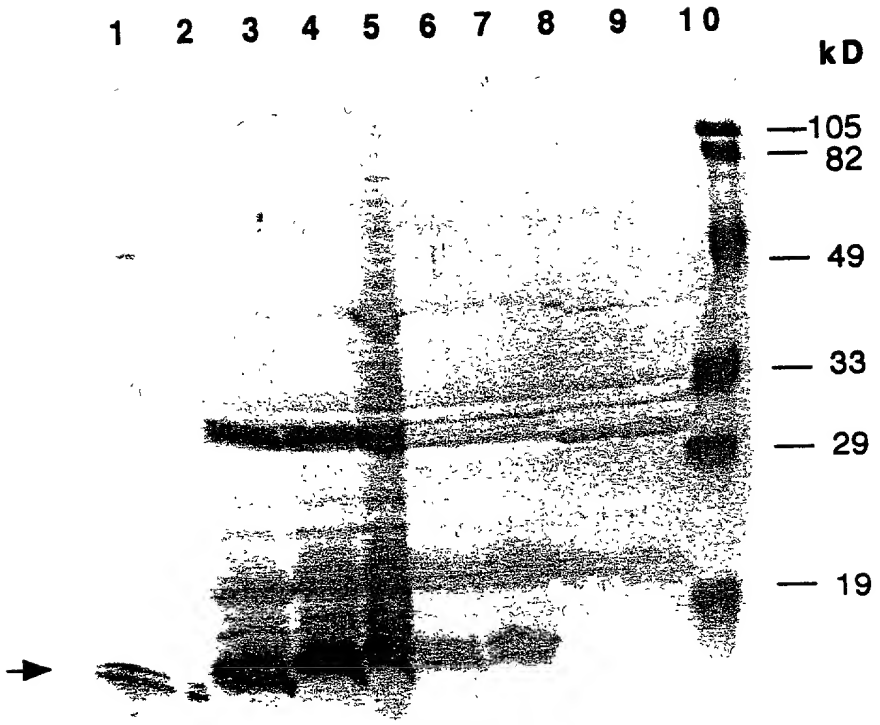


FIG 2.11

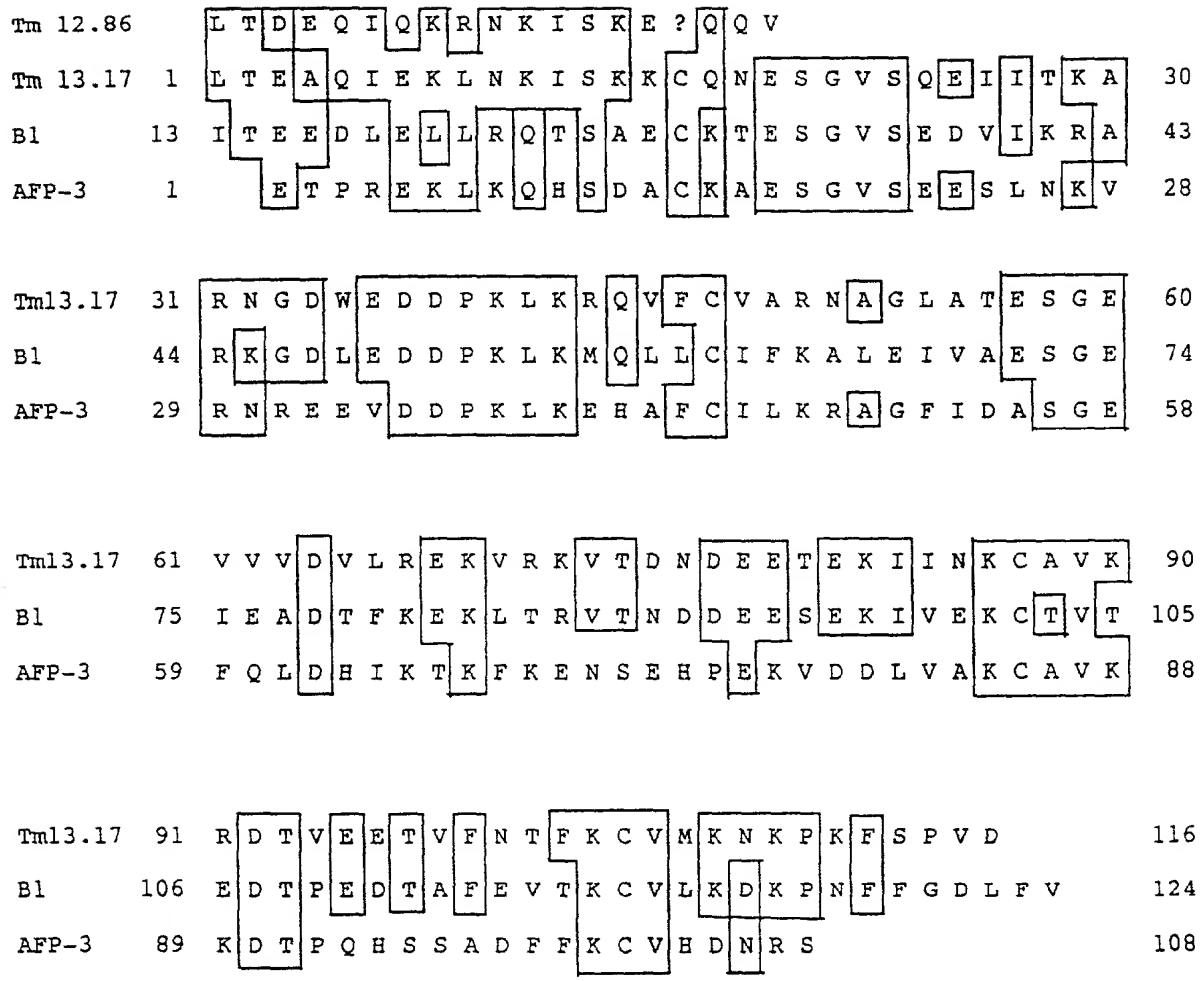

Tm 12.86 L T D E Q I Q K R N K I S K E ? Q Q V
Tm 13.17 1 L T E A Q I E K L N K I S K K C Q N E S G V S Q E I I T K A 30
B1 13 I T E E D L E L L R Q T S A E C K T E S G V S E D V I K R A 43
AFP-3 1 E T P R E K L K Q H S D A C K A E S G V S E E S L N K V 28
Tm13.17 31 R N G D W E D D P K L K R Q V F C V A R N A G L A T E S G E 60
B1 44 R K G D L E D D P K L K M Q L L C I F K A L E I V A E S G E 74
AFP-3 29 R N R E E V D D P K L K E H A F C I L K R A G F I D A S G E 58
Tm13.17 61 V V V D V L R E K V R K V T D N D E E T E K I I N K C A V K 90
B1 75 I E A D T F K E K L T R V T N D D E E S E K I V E K C T V T 105
AFP-3 59 F Q L D H I K T K F K E N S E H P E K V D D L V A K C A V K 88
Tm13.17 91 R D T V E E T V F N T F K C V M K N K P K F S P V D 116
B1 106 E D T P E D T A F E V T K C V L K D K P N F F G D L F V 124
AFP-3 89 K D T P Q H S S A D F F K C V H D N R S 108

FIG 2.12

Doc. No. 1000-0000-000

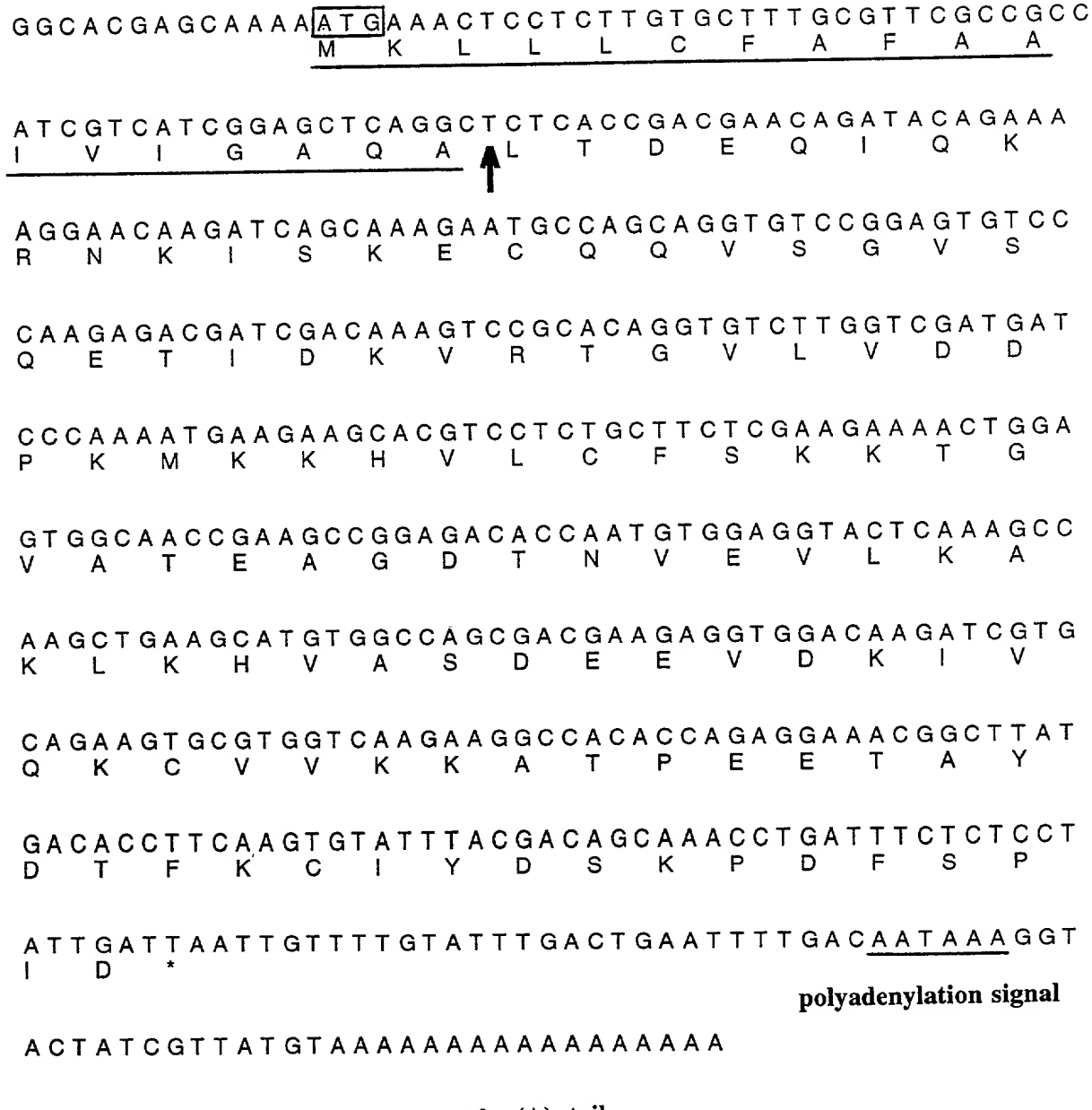


FIG 3.0

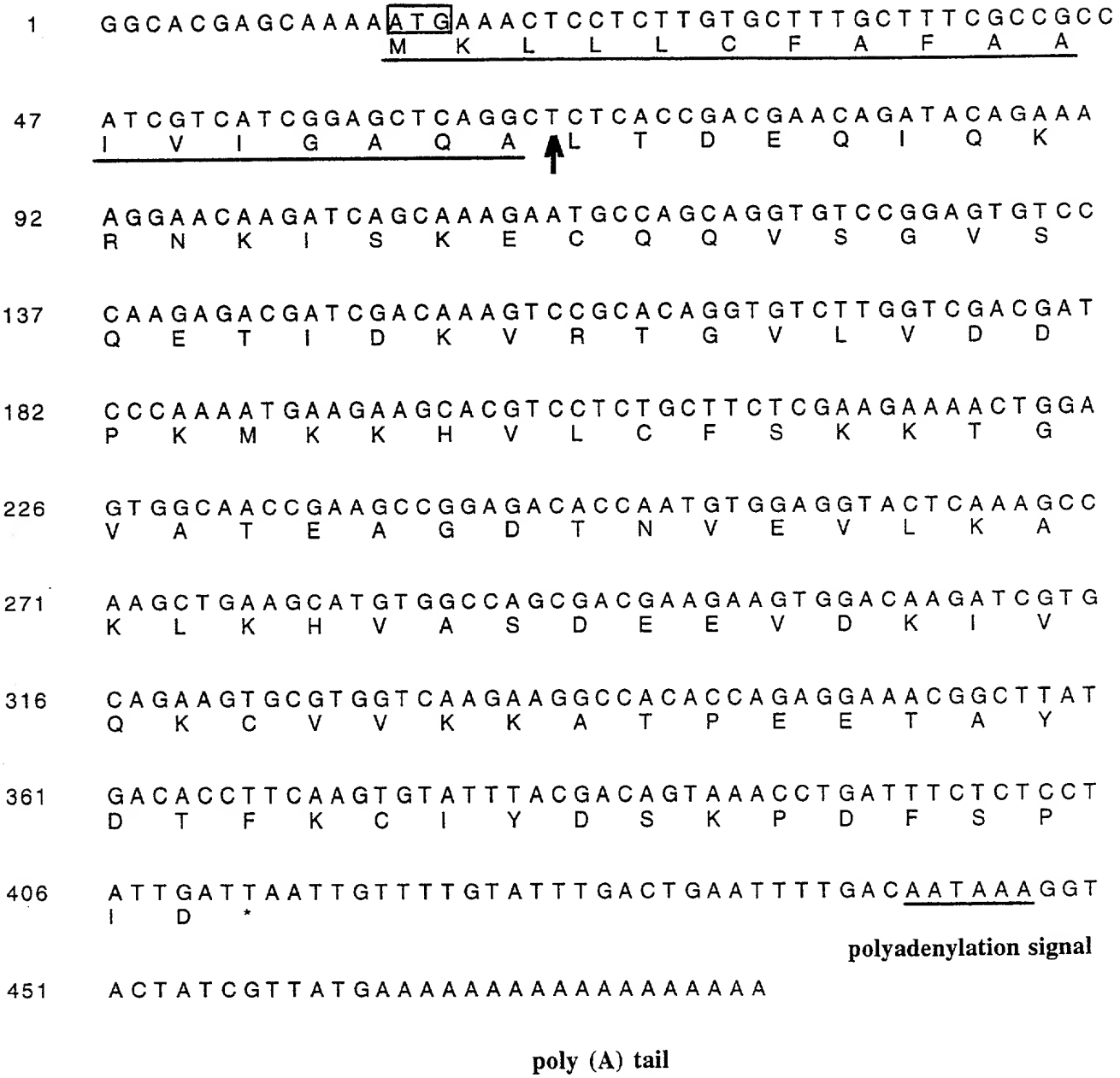

1 GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCTTTCGCCGCC
M K L L L C F A F A A
47 ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
I V I G A Q A L T D E Q I Q K
92 AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCC
R N K I S K E C Q Q V S G V S
137 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
Q E T I D K V R T G V L V D D
182 CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
P K M K K H V L C F S K K T G
226 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
V A T E A G D T N V E V L K A
271 AAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTG
K L K H V A S D E E V D K I V
316 CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
Q K C V V K K A T P E E T A Y
361 GACACCTTCAAGTGTATTTACGACAGTAAACCTGATTTCTCTCCT
D T F K C I Y D S K P D F S P
406 ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
I D *
polyadenylation signal
451 ACTATCGTTATGAAAAAAAAAAAAAAAAA
poly (A) tail

FIG 3.1

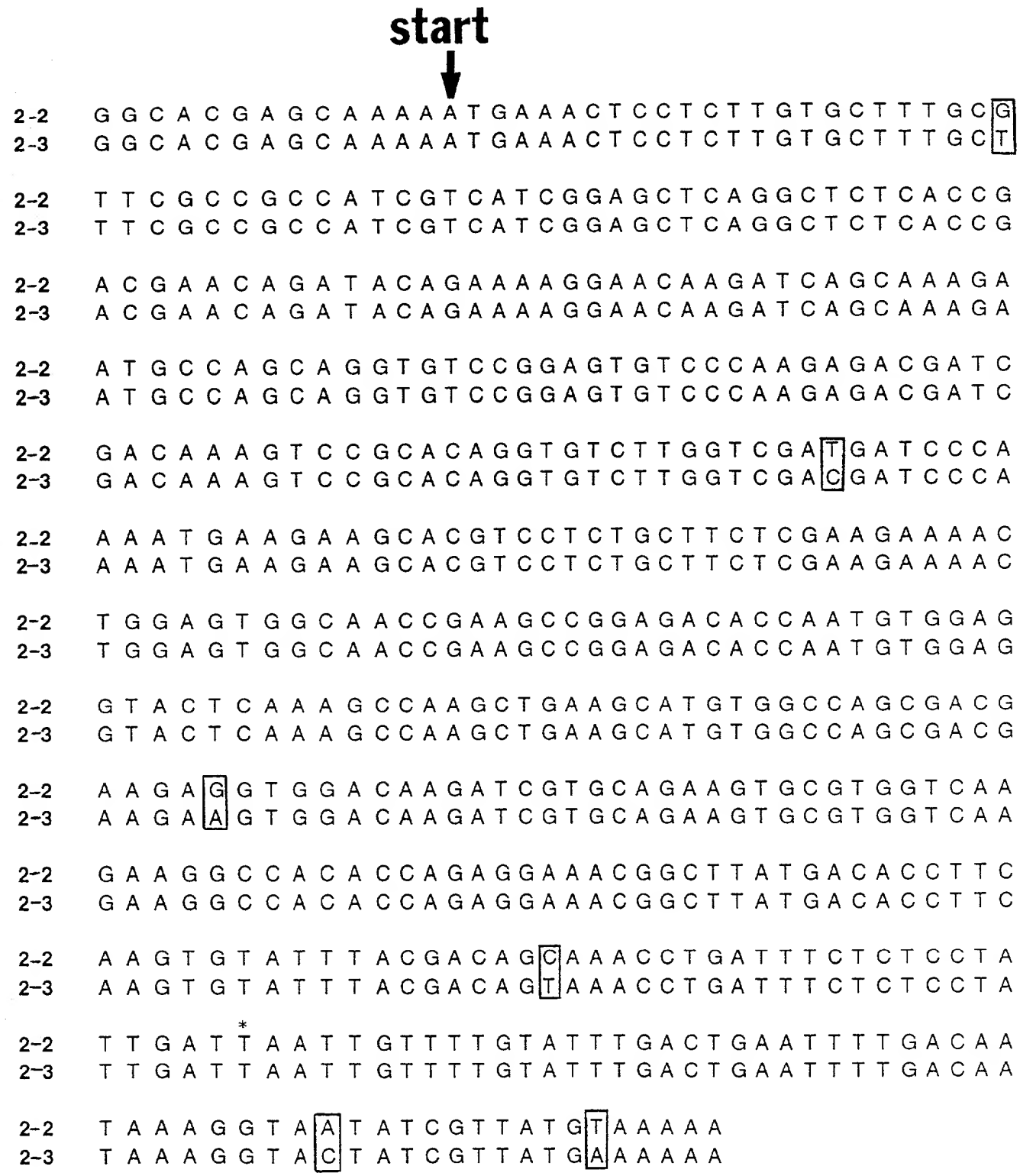

start
2-2 GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCG
2-3 GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCT
2-2 TTCGCCGCCATCGTCATCGGAGCTCAGGCTCTCACCG
2-3 TTCGCCGCCATCGTCATCGGAGCTCAGGCTCTCACCG
2-2 ACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGA
2-3 ACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGA
2-2 ATGCCAGCAGGTGTCCGGAGTGTCCCAAGAGACGATC
2-3 ATGCCAGCAGGTGTCCGGAGTGTCCCAAGAGACGATC
2-2 GACAAAGTCCGCACAGGTGTCTTGGTCGATGATCCCA
2-3 GACAAAGTCCGCACAGGTGTCTTGGTCGACGATCCCA
2-2 AAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAAC
2-3 AAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAAC
2-2 TGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAG
2-3 TGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAG
2-2 GTACTCAAAGCCAAGCTGAAGCATGTGGCCAGCGACG
2-3 GTACTCAAAGCCAAGCTGAAGCATGTGGCCAGCGACG
2-2 AAGAGGTGGACAAGATCGTGCAGAAGTGCGTGGTCAA
2-3 AAGAAGTGGACAAGATCGTGCAGAAGTGCGTGGTCAA
2-2 GAAGGCCACACCAGAGGAAACGGCTTATGACACCTTC
2-3 GAAGGCCACACCAGAGGAAACGGCTTATGACACCTTC
2-2 AAGTGTATTTACGACAGCAAACCTGATTTCTCTCCTA
2-3 AAGTGTATTTACGACAGTAAACCTGATTTCTCTCCTA
2-2 TTGATTAATTGTTTTGTATTTGACTGAATTTTGACAA
2-3 TTGATTAATTGTTTTGTATTTGACTGAATTTTGACAA
2-2 TAAAGGTAATATCGTTATGTAAAAAA
2-3 TAAAGGTACTATCGTTATGAAAAAA

FIG 3.2

Predicted Amino Acid

Composition of 2-2 and 2-3

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
V Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

FIG 3.3

PCT/US96/05095

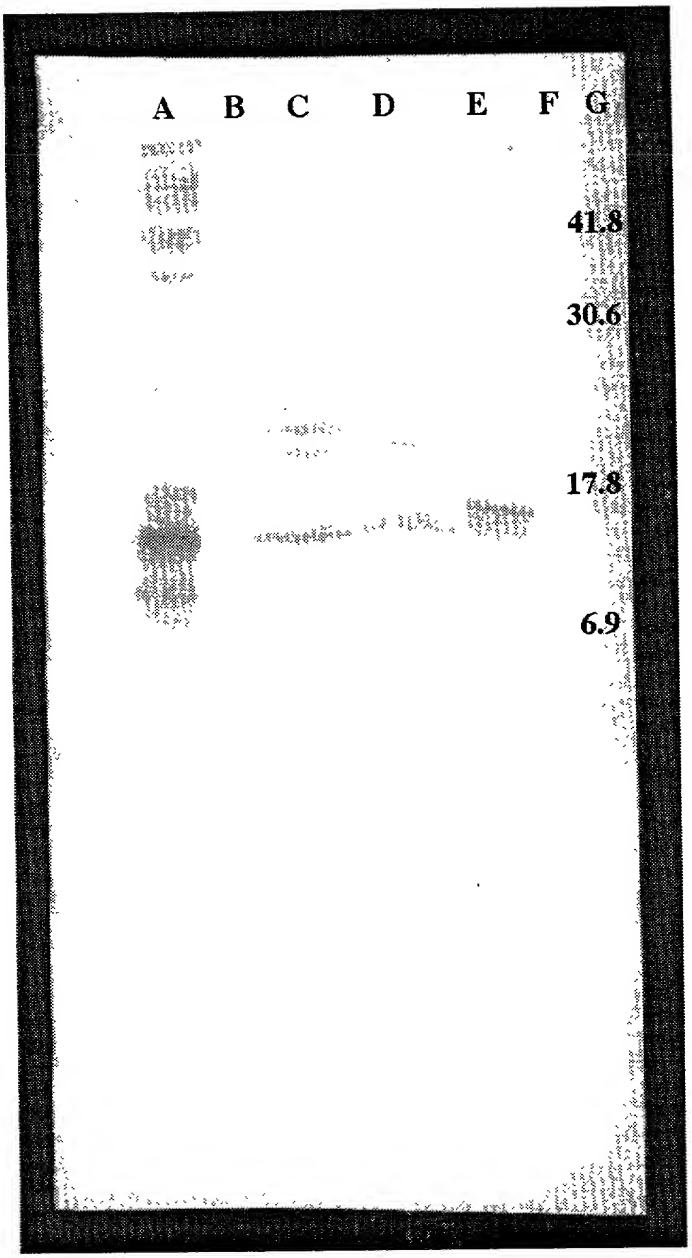


FIG 3.4

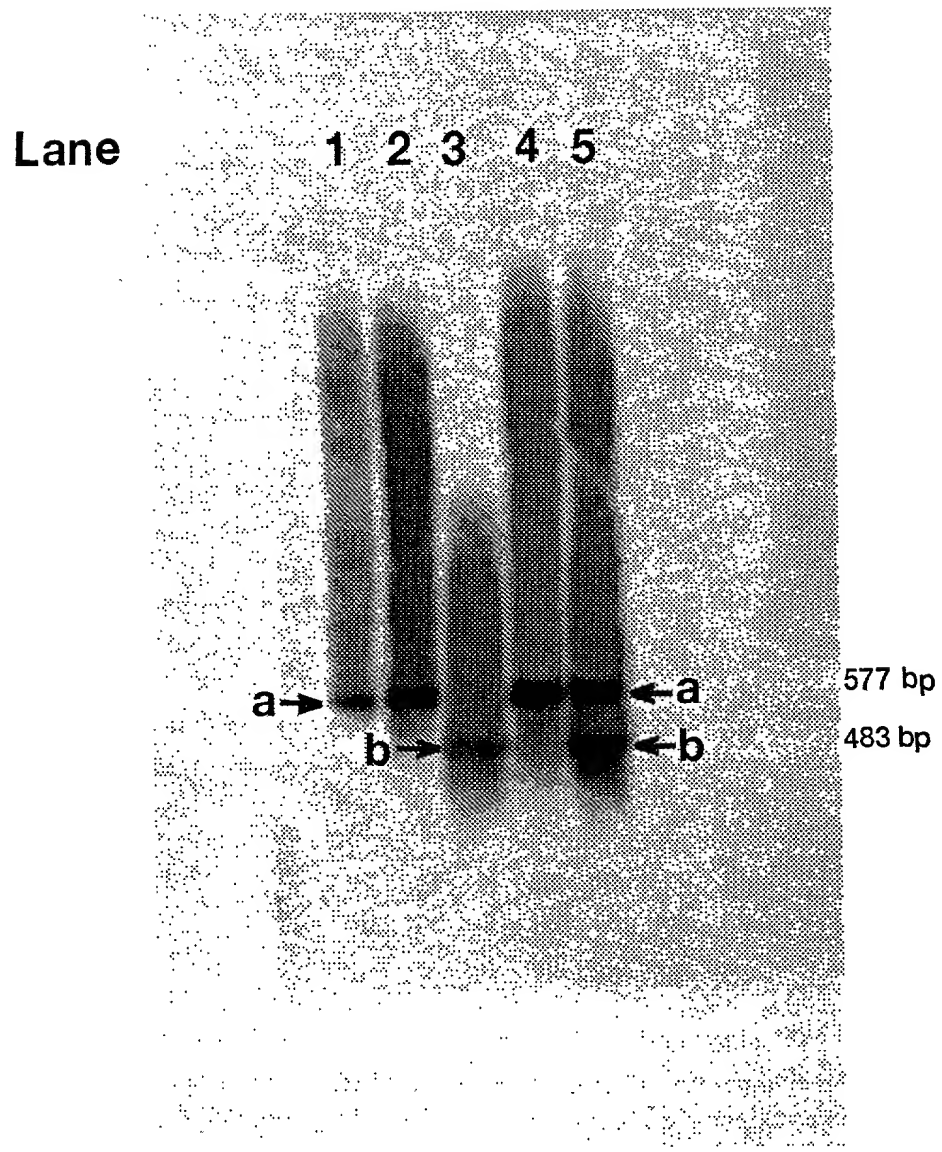


FIG 4.0

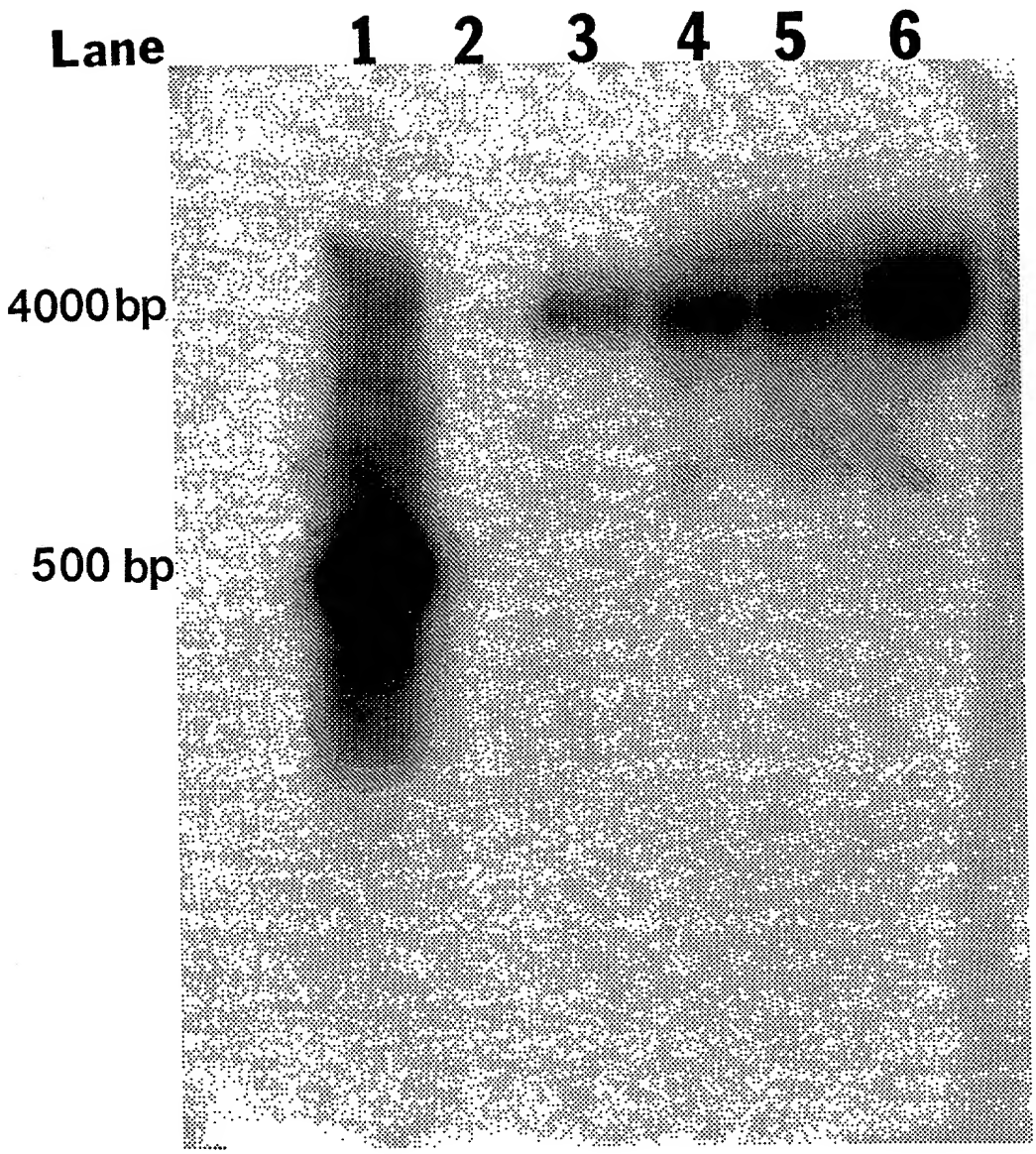


FIG 4.1

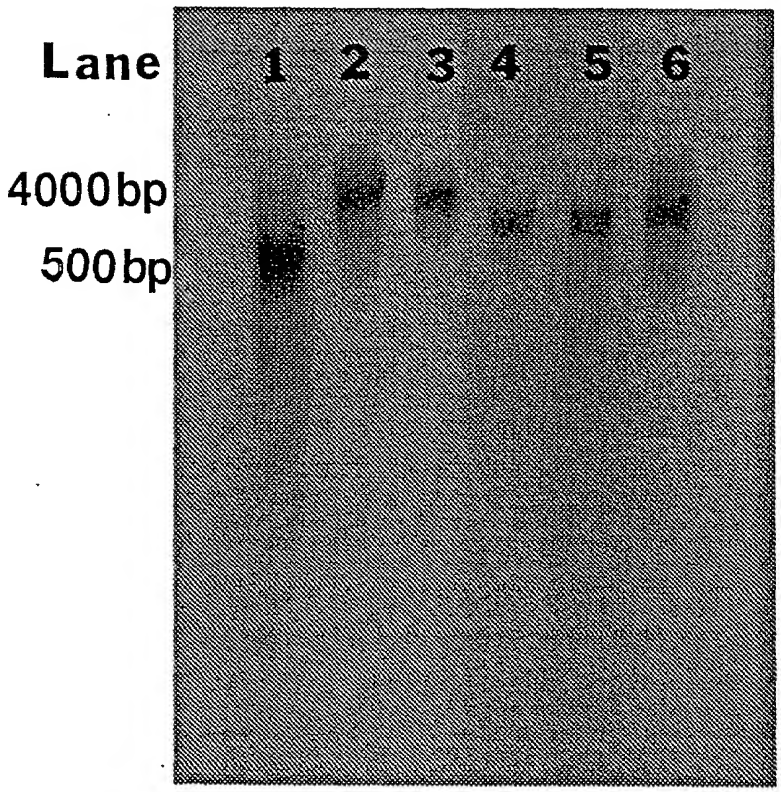
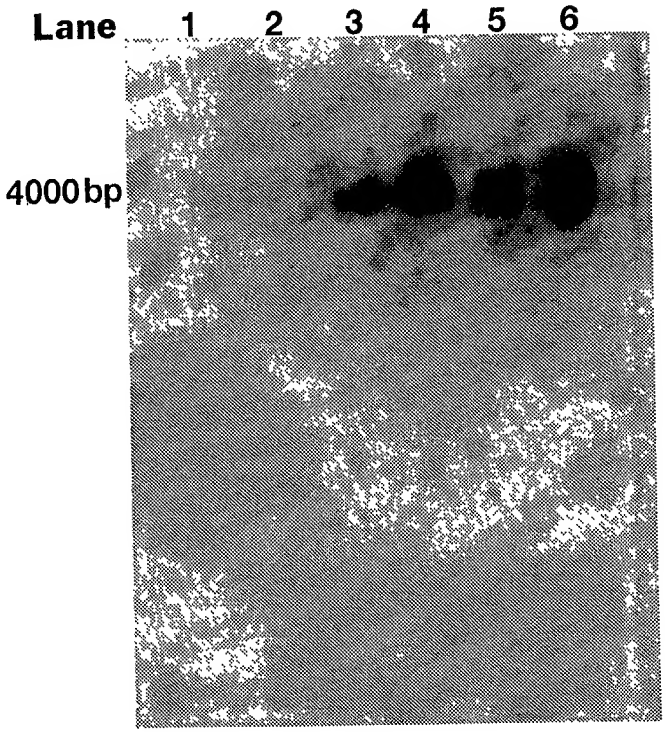


FIG 4.2

TO2030 '96297860

B.

A.



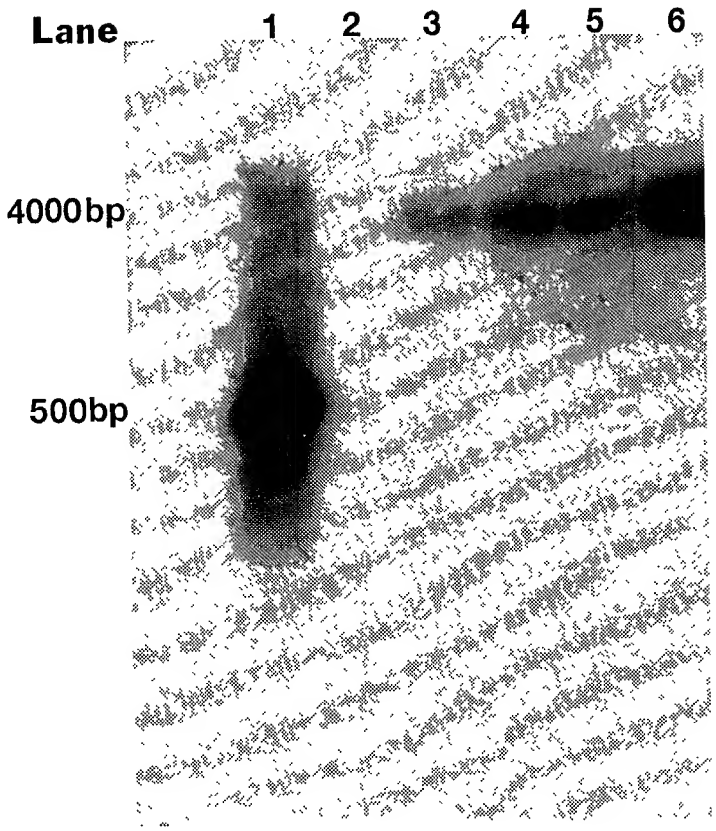


FIG 4.3

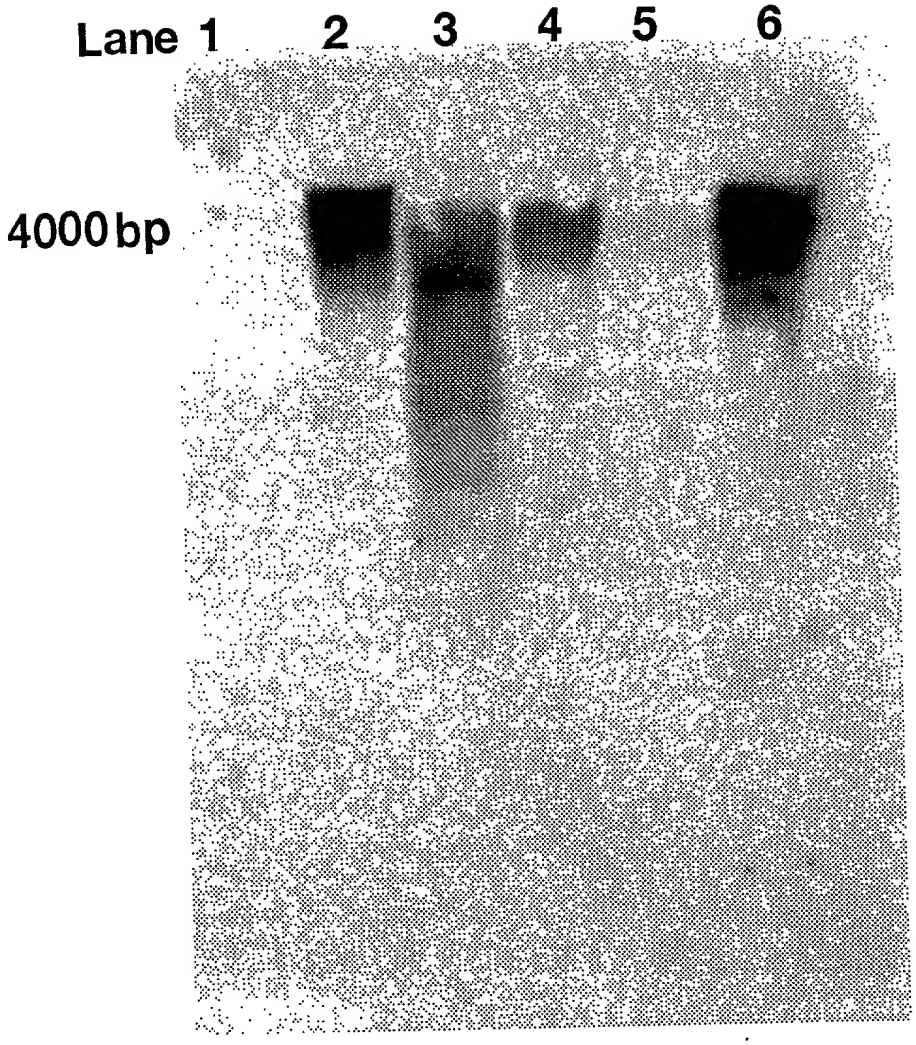
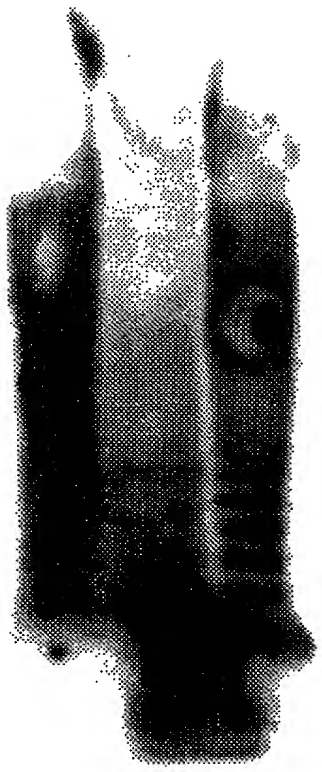


FIG 4.4

Lane 1 2 3 4 5



23130

9416

4361

2322

2027

564

FIG 4.5

Tm 13.17 cDNA

FIG. 4.6 a

B.

Forward Primer

```
2-2        L T D E Q I Q K R N K I S K E C Q Q V S G V S Q E T I D K V R T G V L V
Tm 13.17   L T E A Q I E K L N K I S K K C Q N E S G V S Q E I I T K A R N G D W E
B2         L T E E D L Q L L R Q T S A E C K T E S G A S E A V I K K A R K G D L E
AFP-3        E T P R E K L K Q H S D A C K A E S G V S E E S L N K V R N R E E V

2-2        D D P K M K K H V L C F S K K T G V A T E A G D T N V E V L K A K L K H
Tm 13.17   D D P K L K R Q V F C V A R N A G L A T E S G E V V V D V L R E K V R K
B2         D D P K L K M Q L L C I F K A L E I V A E S G E I E A D T F K E K L T R
AFP-3      D D P K L K E H A F C I L K R A G F I D A S G E F Q L D H I K T K F K E
```

Reverse Primer

```
2-2        V A S  D E E V D K I V Q K C V V K K A T P E E T A Y D T F K C I Y D S
Tm 13.17   V T D N D E E T E K I I N K C A V K R D T V E E T V F N T F K C V M K N
B2         V T N D D E E S E K I V E K C T V T E D T P E D T A F E V T K C V L K D
AFP-3      N S E H P E K V D D L V A K C A V K K D T P Q H S S A D F F K C V H D N

2-2        K P D F S P I D
Tm 13.17   K P K F S P V D
B2         K P N F F G D L F V
AFP-3      R S
```

C.

Primer	percent % composition A	C	G	T	Melting Temperature (°C)
Forward	28.6	14.3	42.9	14.3	44.0
Reverse	25.0	31.3	6.3	37.5	44.0

FIG 4.6

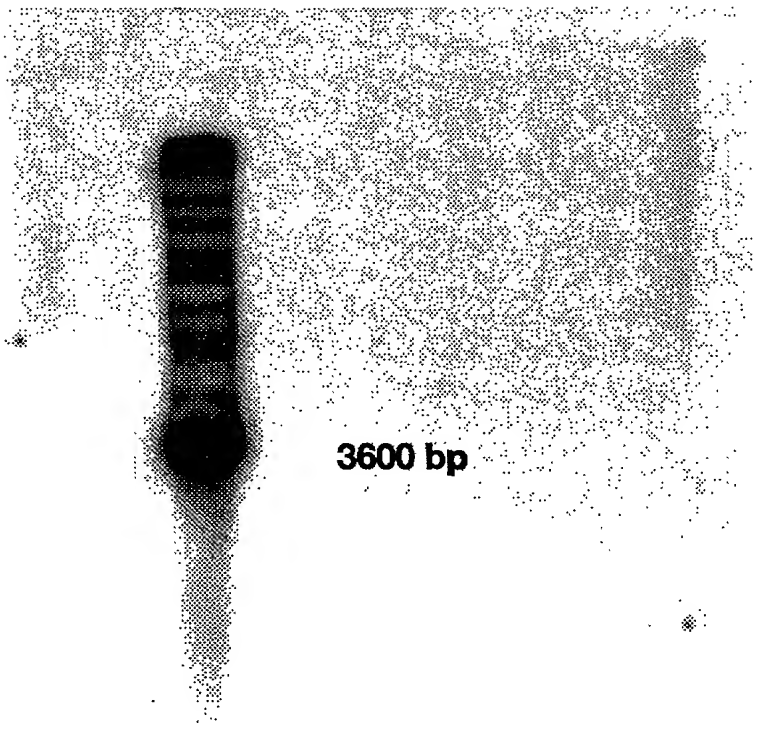


FIG 4.7

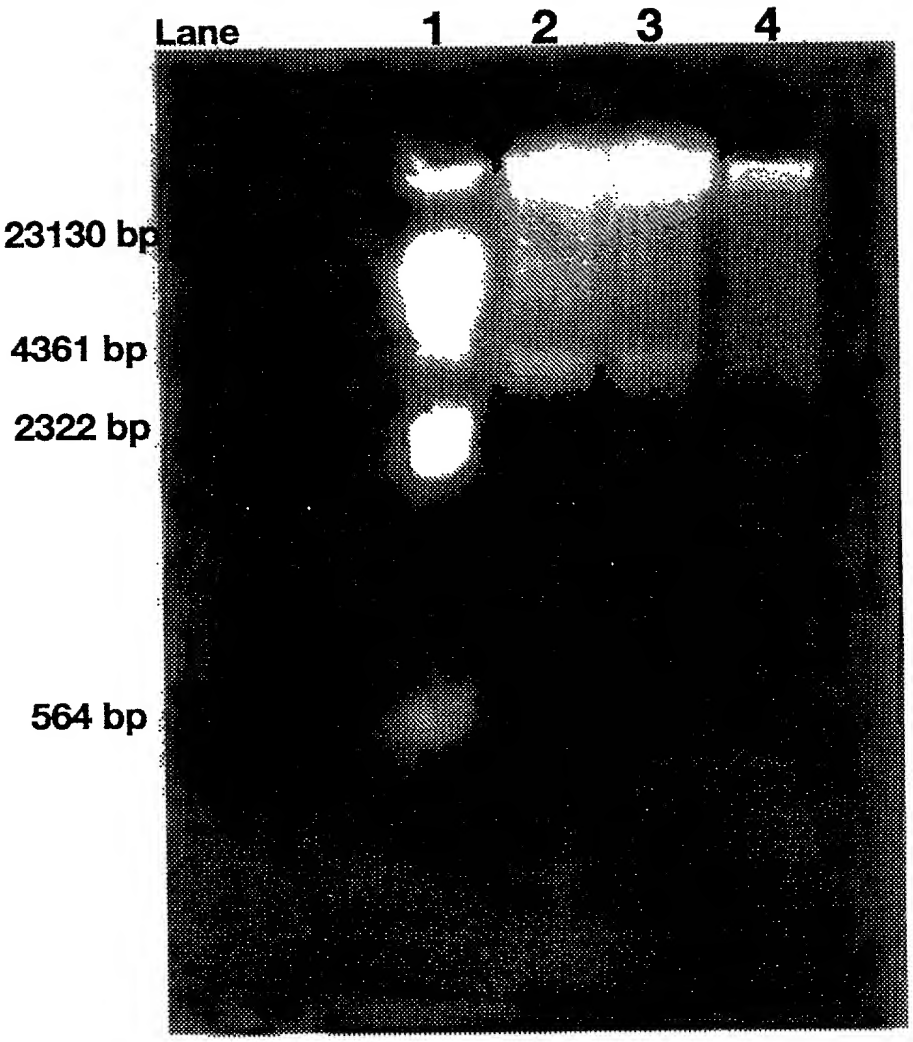


FIG 4.8

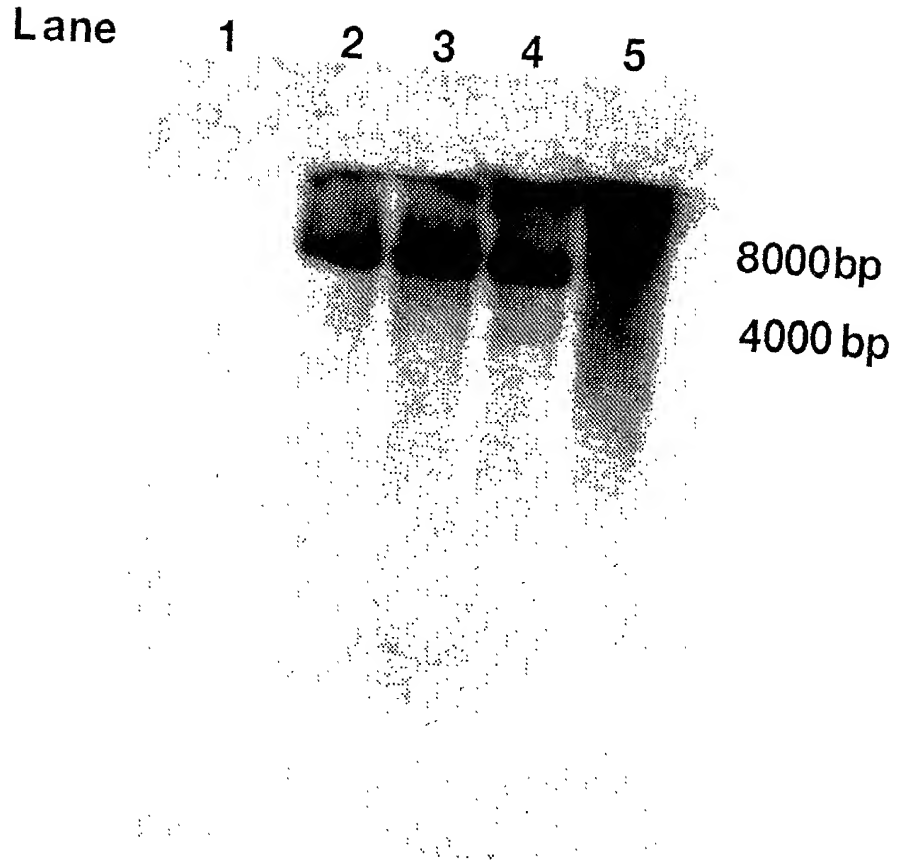
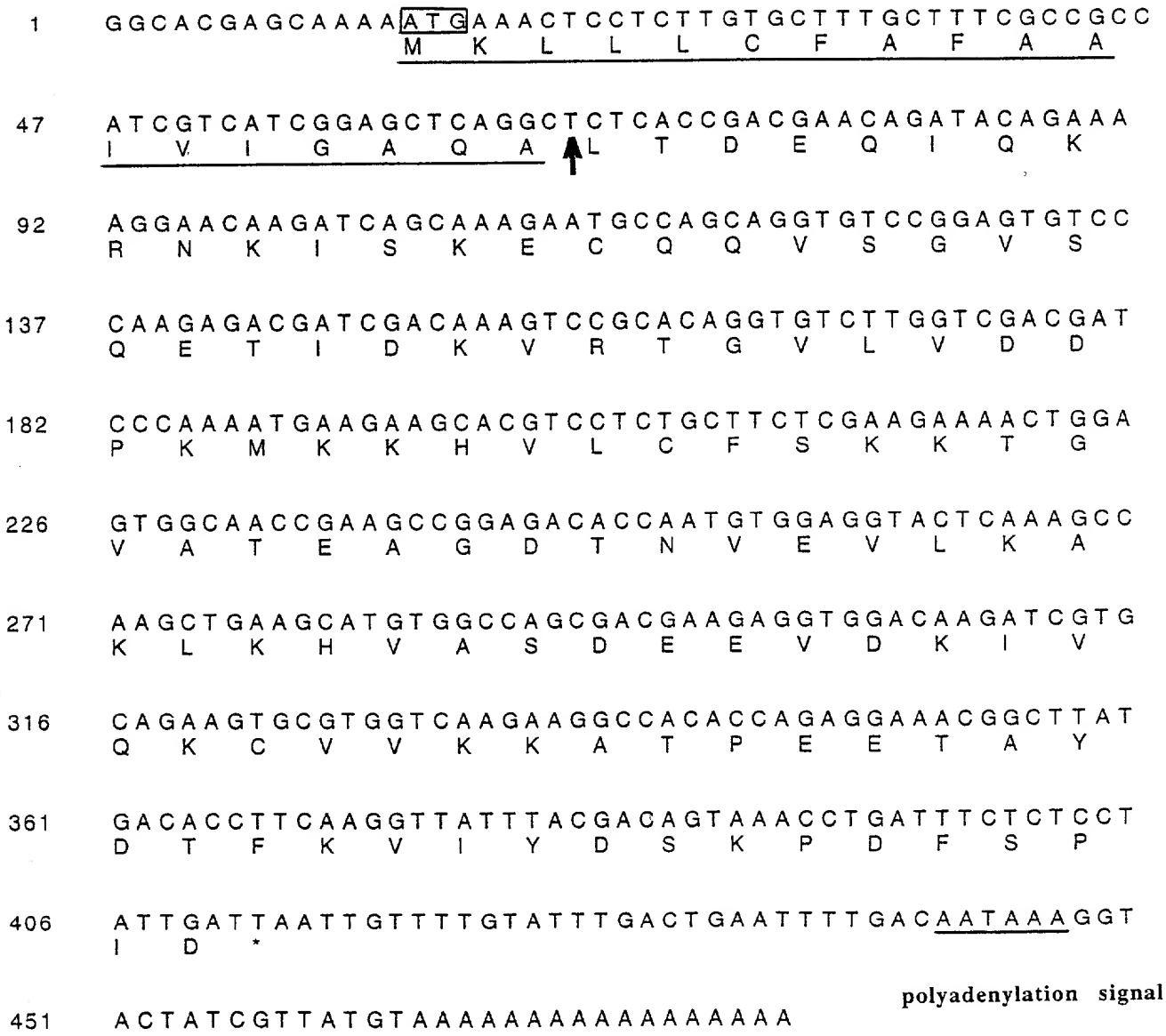


FIG 4.9

Printed: 05/05/1999



poly (A) tail

FIG. 4.10 a

Analysis	Whole Protein
Molecular Weight	12839.70 m.w.
Length	115
1 microgram =	77.883 pMoles
Molar Extinction coefficient	2920±5%
1 A(280) =	4.40 mg/ml
Isoelectric Point	7.14
Charge at pH 7	0.16

Predicted Amino Acid

Composition of 3–4

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHY.CDE)	47	46.41	40.87
Acidic (DE)	20	18.91	17.39
Basic (KR)	20	20.41	17.39
Polar (NCQSTY)	29	24.55	25.22
Hydrophobic (AILFWV)	35	28.04	30.43
A Ala	6	3.32	5.22
C Cys	3	2.41	2.61
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.99	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.09	7.83
V Val	15	11.58	13.04
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

PROPRIETARY INFORMATION

FIG. 4.10 b

SUBSTITUTE SHEET (RULE 26)

```
1    GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCTTTCGCCGCC
                M  K  L  L  L  C  F  A  F  A  A

47   ATCGTCATCGGAGCTCAGGCTCTCACCGATGAACAGATACAGAAA
     I  V  I  G  A  Q  A  L  T  D  E  Q  I  Q  K

92   AGGAACAAGATCAGCAAAGAATGCCAGCAGGAGTCCGGAGTGTCC
     R  N  K  I  S  K  E  C  Q  Q  E  S  G  V  S

137  CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
     Q  E  T  I  D  K  V  R  T  G  V  L  V  D  D

182  CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAGAACTGGA
     P  K  M  K  K  H  V  L  C  F  S  K  R  T  G

226  GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
     V  A  T  E  A  G  D  T  N  V  E  V  L  K  A

271  AAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTG
     K  L  K  H  V  A  S  D  E  E  V  D  K  I  V

316  CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
     Q  K  C  V  V  K  K  A  T  P  E  E  T  A  Y

361  GACACCTTCAAGTGTATTTACGACAGTAAACCTGATTTCTCTCCT
     D  T  F  K  V  I  Y  D  S  K  P  D  F  S  P

406  ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
     I  D  *
                                        polyadenylation signal

451  ACTATCGTTATGAAAAAAAAAAAAAAAAAA
```

poly (A) tail

FIG. 4.11 a

Predicted Amino Acid

Composition of 3-9

Analysis	Whole Protein
Molecular Weight	12871.80 m.w.
Length	115
1 microgram =	77.689 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.23 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	48	47.31	41.74
Acidic (DE)	20	18.86	17.39
Basic (KR)	20	20.57	17.39
Polar (NCQSTY)	30	25.29	26.09
Hydrophobic (AILFWV)	34	27.20	29.57
A Ala	6	3.31	5.22
C Cys	4	3.21	3.48
D Asp	11	9.84	9.57
E Glu	9	9.03	7.83
F Phe	3	3.43	2.61
G Gly	4	1.77	3.48
H His	2	2.13	1.74
I Ile	6	5.28	5.22
K Lys	17	16.93	14.78
L Leu	5	4.40	4.35
M Met	1	1.02	0.87
N Asn	2	1.77	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.97	5.22
R Arg	3	3.64	2.61
S Ser	7	4.74	6.09
T Thr	9	7.07	7.83
V Val	14	10.78	12 17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

FIG. 4.11 b

EP 0 599 528 A2

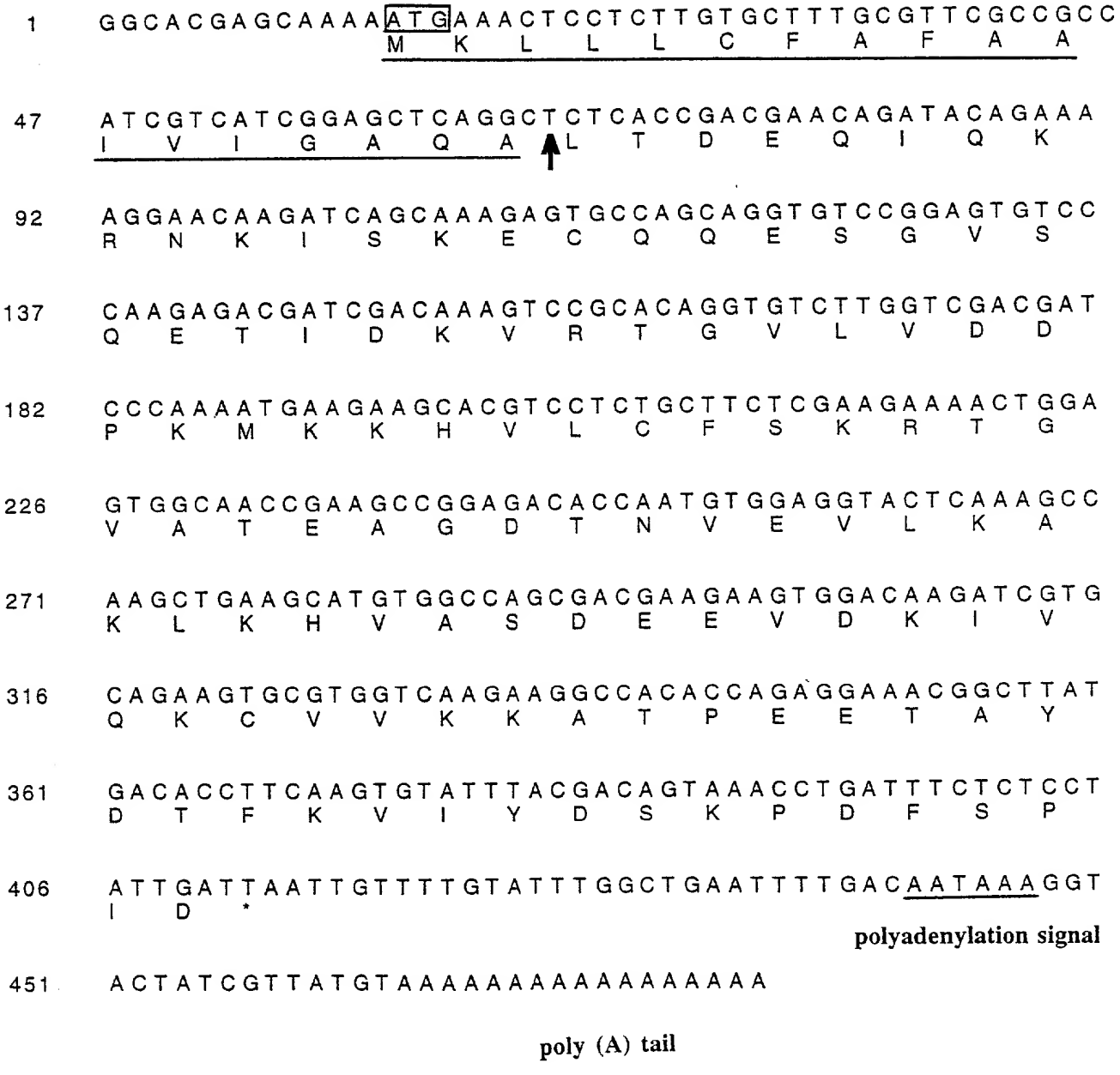


FIG. 4.12 a

Predicted Amino Acid

Composition of 7–5

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
V Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

FIG. 4.12 b

EP 0 968 268 A2

EP 2 090 967 A2

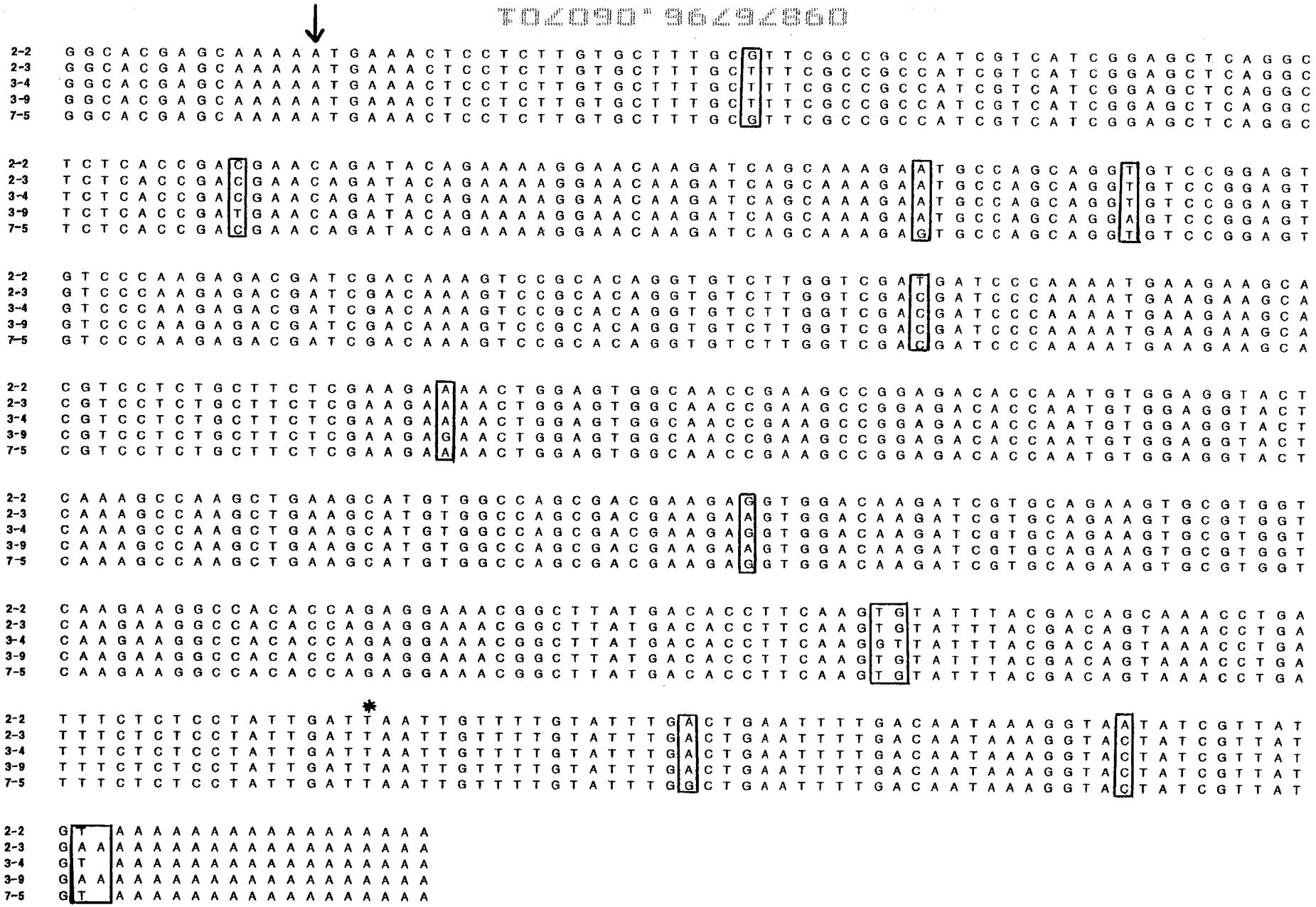
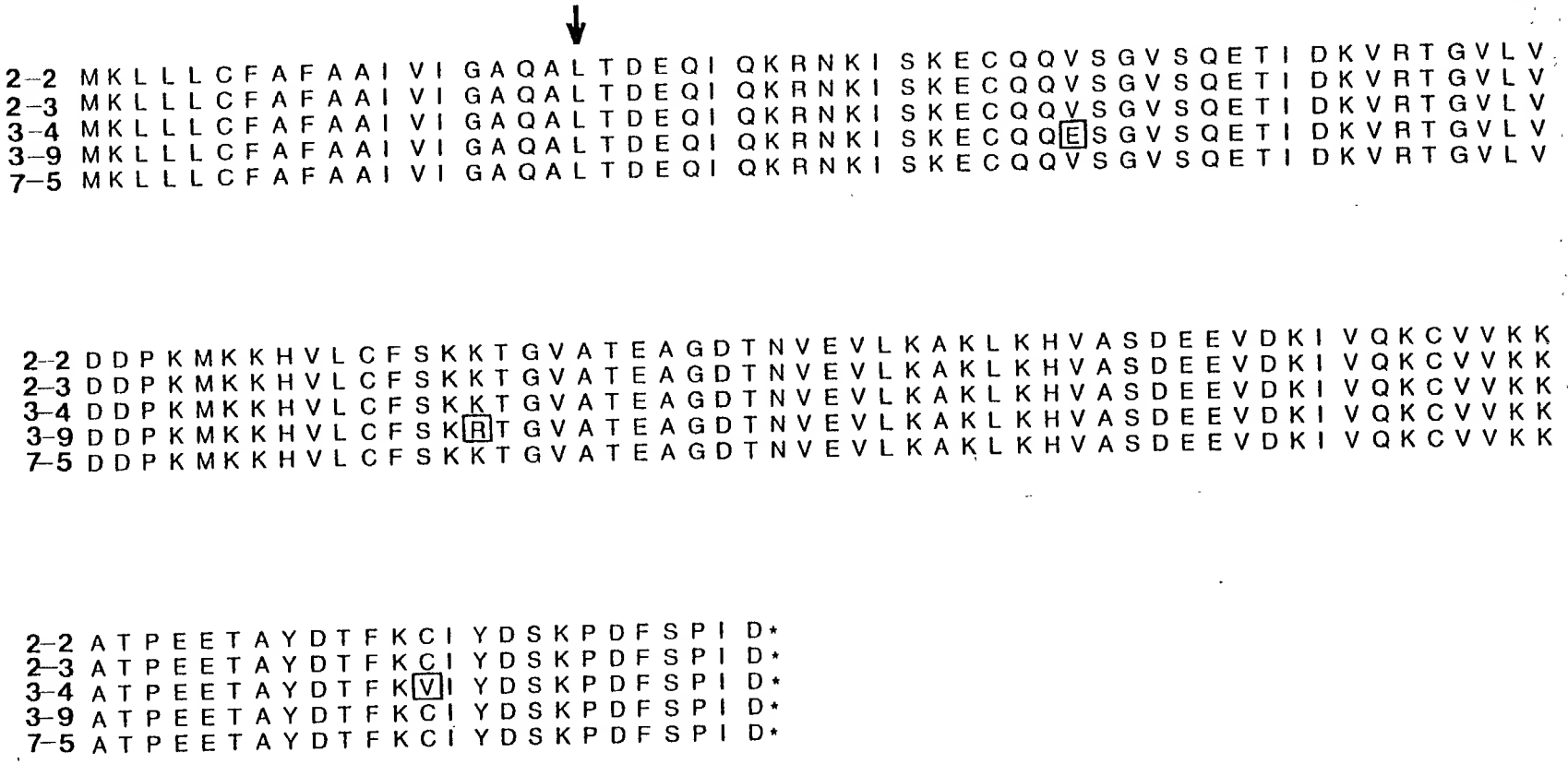


FIG. 4.13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2–3 MKLLLCFAFAAIVIGAQALTDEQIQKRNKISKECQQVSGVSQETIDKVRTGVLV
3–4 MKLLLCFAFAAIVIGAQALTDEQIQKRNKISKECQQVSGVSQETIDKVRTGVLV
3–9 MKLLLCFAFAAIVIGAQALTDEQIQKRNKISKECQQESGVSQETIDKVRTGVLV
7–5 MKLLLCFAFAAIVIGAQALTDEQIQKRNKISKECQQVSGVSQETIDKVRTGVLV
2–2 DDPKMKKHVLCFSKKTGVATEAGDTNVEVLKAKLKHVASDEEVDKIVQKCVVKK
2–3 DDPKMKKHVLCFSKKTGVATEAGDTNVEVLKAKLKHVASDEEVDKIVQKCVVKK
3–4 DDPKMKKHVLCFSKKTGVATEAGDTNVEVLKAKLKHVASDEEVDKIVQKCVVKK
3–9 DDPKMKKHVLCFSKRTGVATEAGDTNVEVLKAKLKHVASDEEVDKIVQKCVVKK
7–5 DDPKMKKHVLCFSKKTGVATEAGDTNVEVLKAKLKHVASDEEVDKIVQKCVVKK
2–2 ATPEETAYDTFKCIYDSKPDFSPID*
2–3 ATPEETAYDTFKCIYDSKPDFSPID*
3–4 ATPEETAYDTFKVIYDSKPDFSPID*
3–9 ATPEETAYDTFKCIYDSKPDFSPID*
7–5 ATPEETAYDTFKCIYDSKPDFSPID*

FIG. 4.14

TO2090 "96292860

(kDa) (% mole)

	MW	AA	Cys	Pro	Phe	Ile	Val	Met	Leu	% most hydrophobic	Gly	Ala	Tyr	His	Trp	Asx	Glx	Arg	Lys	Ser	Thr	% most hydrophilic
Tm 12.86	12.86	117	3.2	3.0	3.4	4.4	8.5	2.0	4.4	28.9	3.1	3.9	3.8	3.2	ND	10.7	15.0	3.6	14.9	6.8	6.3	57.3
Tm 13.17	13.17	116	3.13	2.21	4.47	5.16	10.5	1.0	4.3	29.11	1.73	3.24	0	0	1.41	0	0	7.12	15.6	3.31	6.14	32.14
2-2	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.08	32.23
2-3	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.08	32.23
3-4	12.84	115	2.41	3.02	3.44	5.29	11.6	1.02	4.41	28.04	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.09	32.24
3-9	12.87	115	3.21	3.02	3.43	5.28	10.8	1.02	4.40	27.20	1.77	3.31	2.54	2.13	0	0	0	3.64	16.9	4.74	7.07	32.38
7-5	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.08	32.23

FIG. 4.15

TO2090 96292860

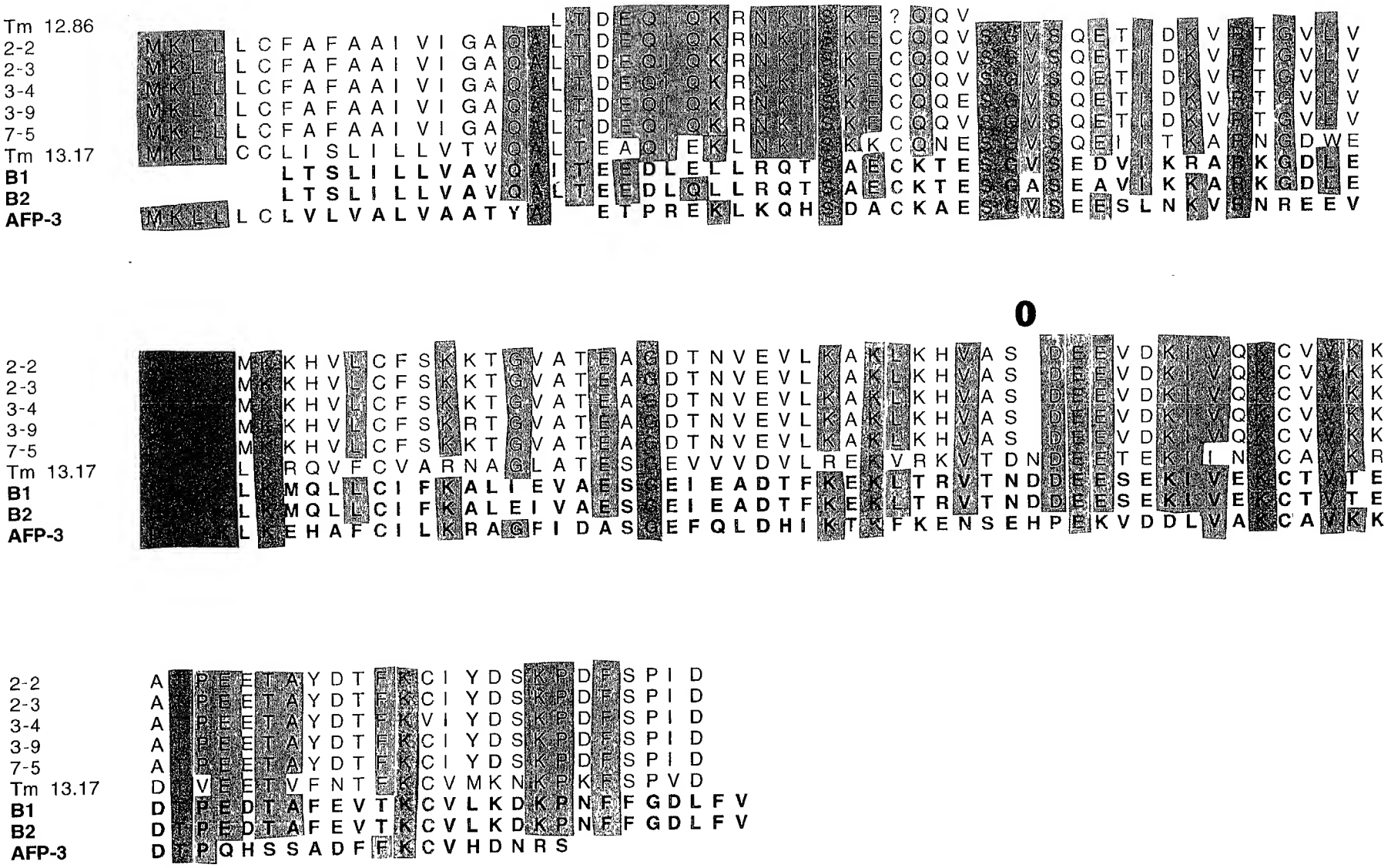
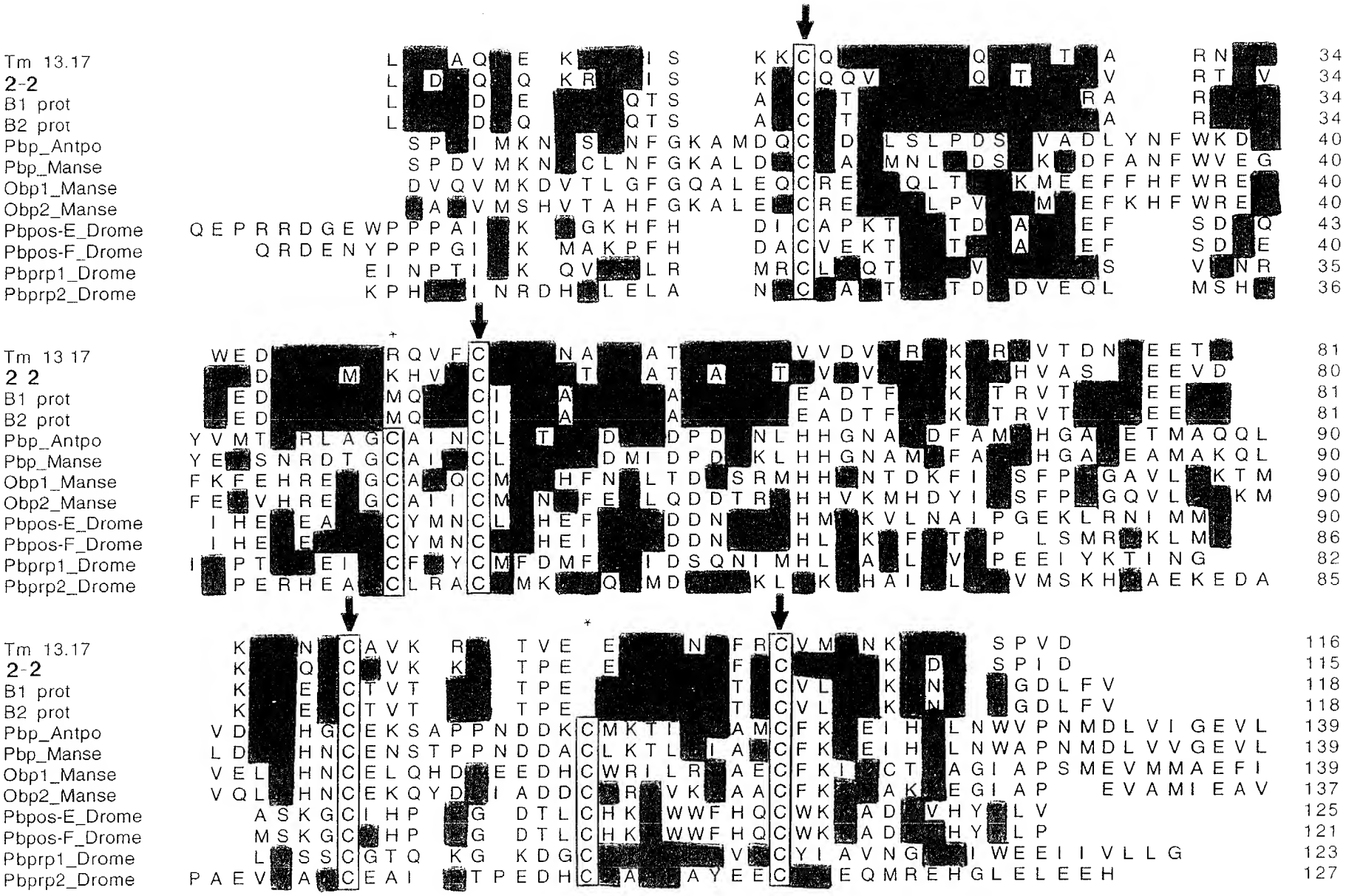


FIG. 4.16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B1 prot
B2 prot
Pbp_Antpo
Pbp_Manse
Obp1_Manse
Obp2_Manse
Pbpos-E_Drome
Pbpos-F_Drome
Pbprp1_Drome
Pbprp2_Drome

FIG. 4.17

FIG. 4.18

NUCLEOTIDE SEQUENCES

PERCENT SIMILARITY

	1 2-2	2 2-3	3 3-4	4 3-9	5 7-5	6 Tm13.17	7 B1	8 AFP-3	9 YL-1	10 DAFP-1A		
1		99.1	97.3	98.4	98.2	50.4	42	42.4	20.7	20.9	1	2-2
2	0.9		99	99	99	51.5	43.2	42.7	20.2	21.3	2	2-3
3	1.6	60		98	98	50.3	42	42.1	20	20	3	3-4
4	1.6	20	80		98.4	51.3	43.7	43.6	20.4	22	4	3-9
5	1.8	25	75	45		50.4	42.5	42.9	22.2	23.1	5	7-5
6	36.9	36.4	37.2	36.4	37.1		57.2	37.4	23.3	21	6	Tm 13.17
7	39.9	41.2	41.7	40.7	41.2	30.2		39.3	21.9	22.1	7	B1
8	41.8	42.4	43.1	42.3	42.3	45.3	49.4		21.8	23.1	8	AFP-3
9	48.7	58.6	52	51.2	51.9	61.7	62.2	58.2		45.6	9	YL-1
10	51.4	62.7	46.4	46.5	46.5	60	58.1	61.8	35.5		10	DAFP-1A
	1	2	3	4	5	6	7	8	9	10		

PERCENT DIVERGENCE

AMINO ACID SEQUENCES

PERCENT SIMILARITY

	1 2-2	2 2-3	3 3-4	4 3-9	5 7-5	6 Tm13.17	7 B1	8 AFP-3	9 YL-1	10 DAFP-1A		
1		100	99.1	99.1	100	51.3	37.4	35.2	11.6	12	1	2-2
2	0		99.1	99.1	100	51.3	37.4	35.2	11.6	12	2	2-3
3	0.9	0.9		98.3	99.1	50.4	36.5	34.3	11.6	12	3	3-4
4	0.9	0.9	1.7		99.1	51.3	37.4	36.1	10.7	12	4	3-9
5	0	0	0.9	0.09		51.3	37.4	35.2	11.6	12	5	7-5
6	46.1	46.1	47	46.1	46.1		47.4	39.8	13.4	13.9	6	Tm 13.17
7	59.1	59.1	60	59.1	59.1	51.7		37	11.6	11.1	7	B1
8	61.7	61.7	62.6	60.7	61.7	60.2	63		10.2	8.3	8	AFP-3
9	86.7	86.7	85.7	85.7	85.7	86.8	84.2	87.5		55.6	9	YL-1
10	88.3	88.3	86.4	84.5	85.4	89.1	82.7	90.5	40.2		10	DAFP-1A
	1	2	3	4	5	6	7	8	9	10		

PERCENT DIVERGENCE

FIG 4.19

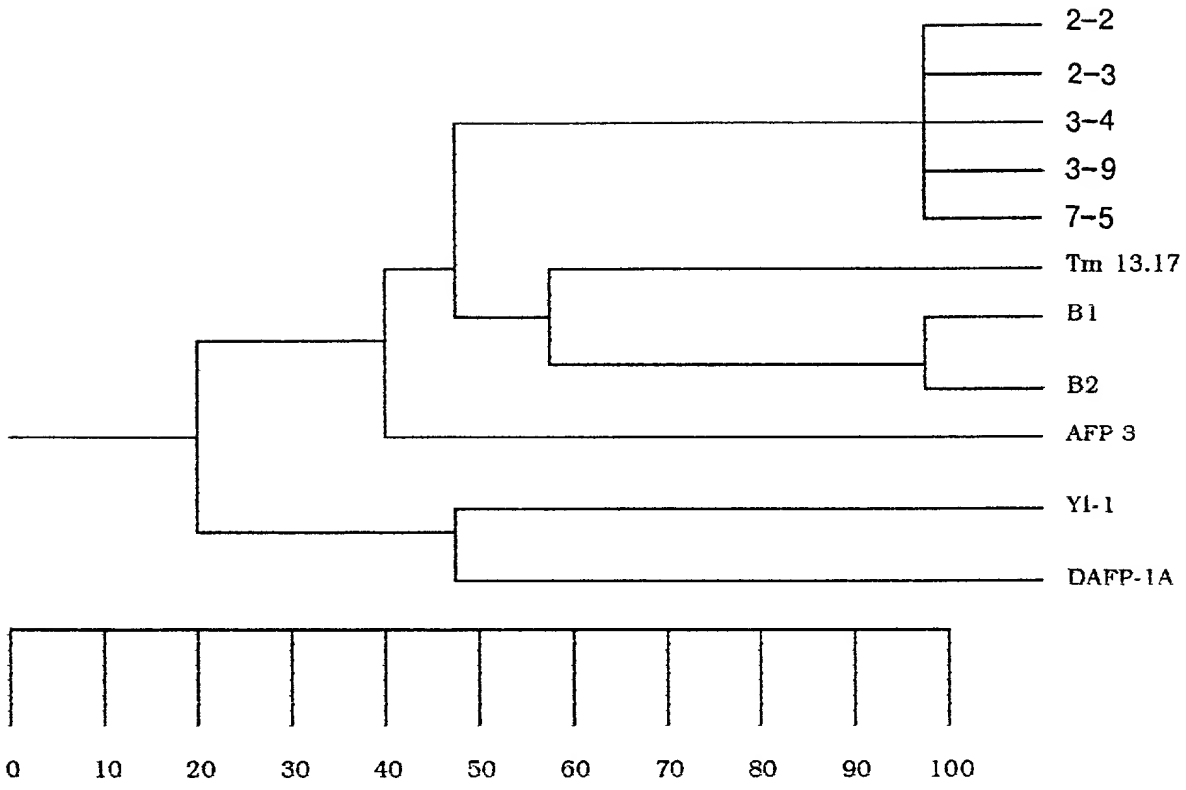

2-2
2-3
3-4
3-9
7-5
Tm 13.17
B1
B2
AFP 3
Yl-1
DAFP-1A
0
10
20
30
40
50
60
70
80
90
100
% Nucleic Acid Identity

FIG 4.20

Electronic Copy - Do Not Delete

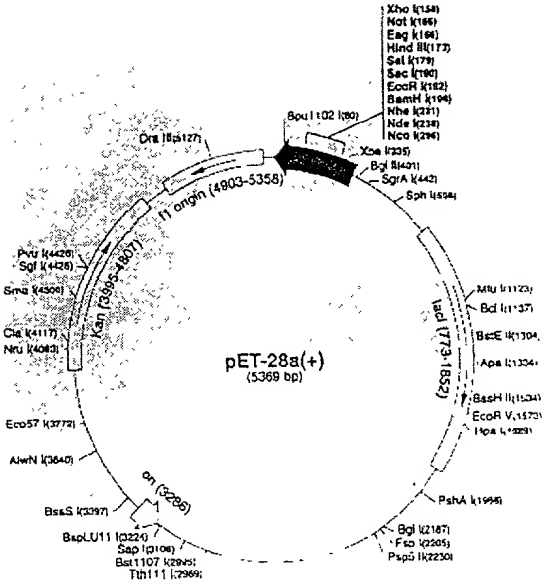


FIG. 5.0

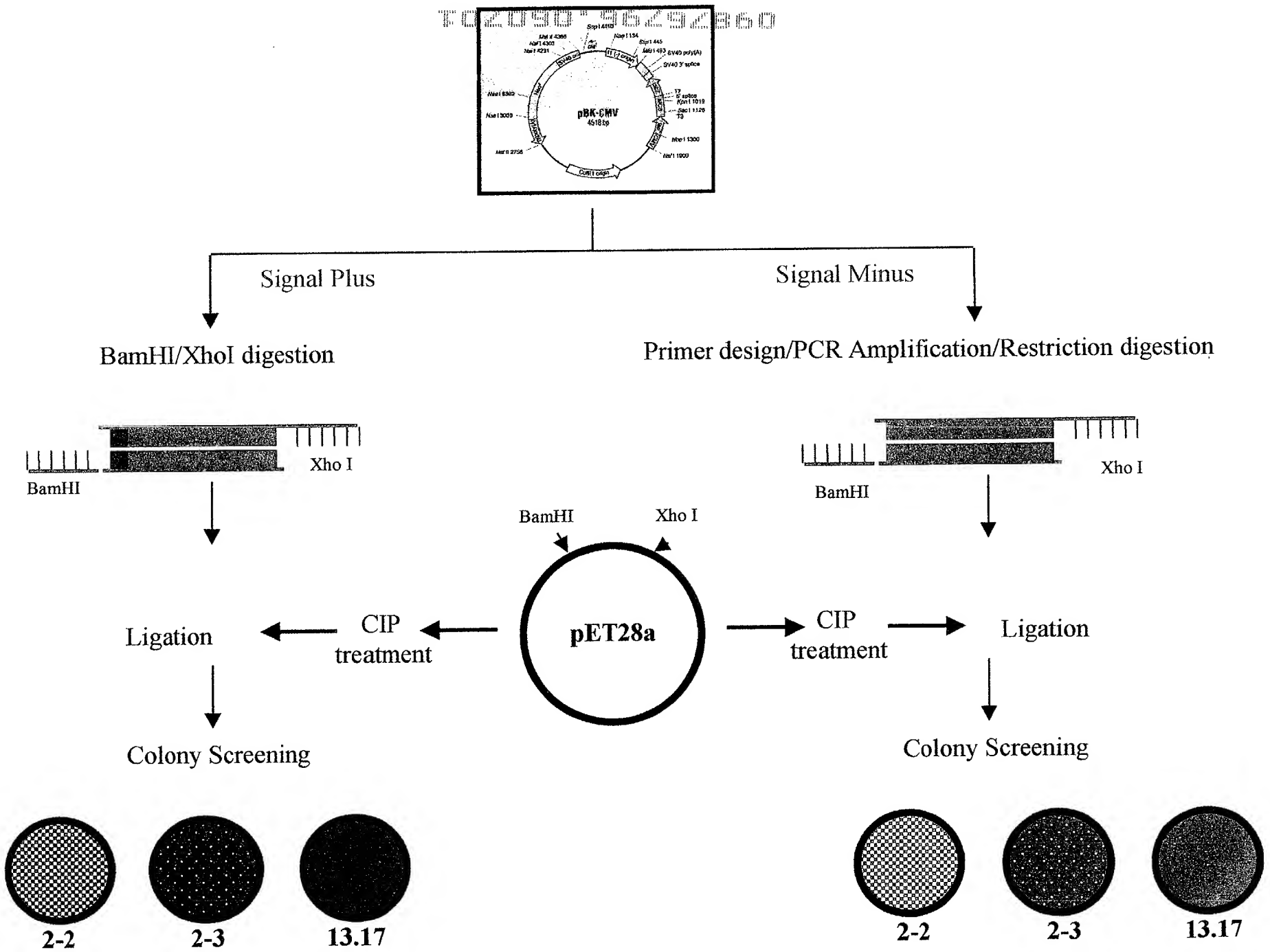

pBK-CMV
Signal Plus
Signal Minus
BamHI/XhoI digestion
Primer design/PCR Amplification/Restriction digestion
BamHI
Xho I
BamHI
Xho I
BamHI
Xho I
Ligation
CIP treatment
pET28a
CIP treatment
Ligation
Colony Screening
Colony Screening
2-2
2-3
13.17
2-2
2-3
13.17

FIG. 5.1

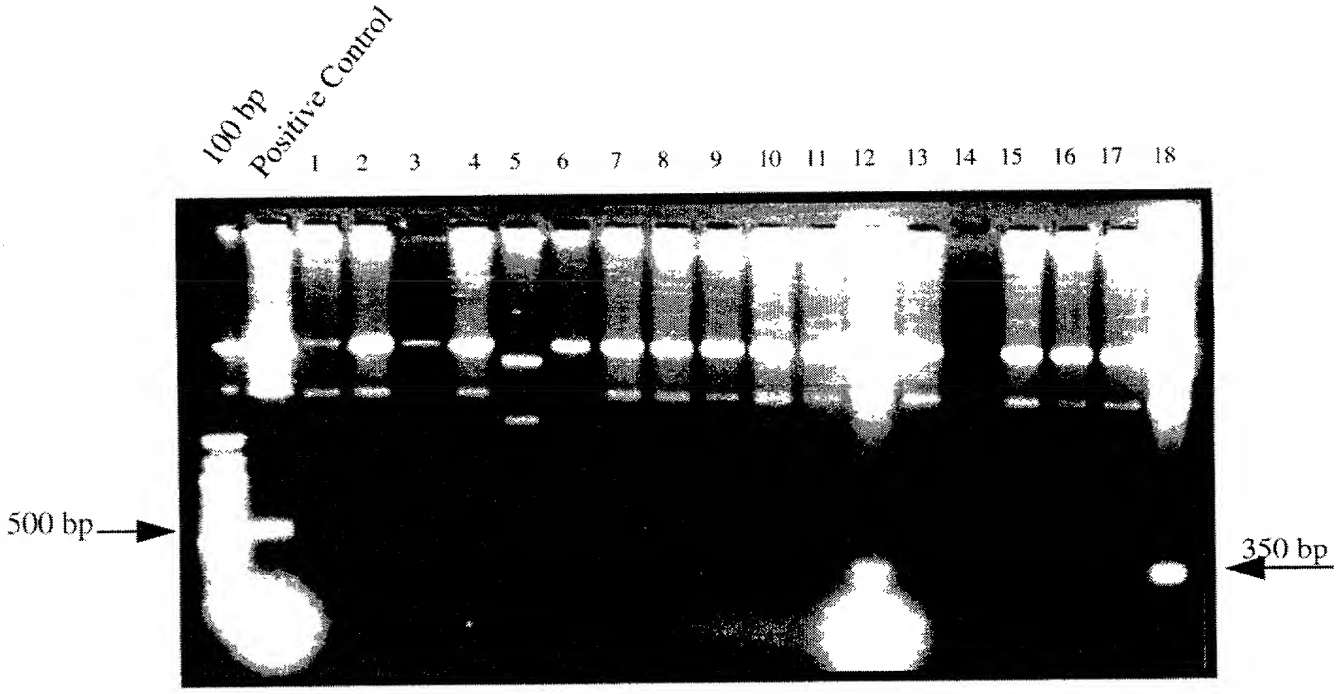
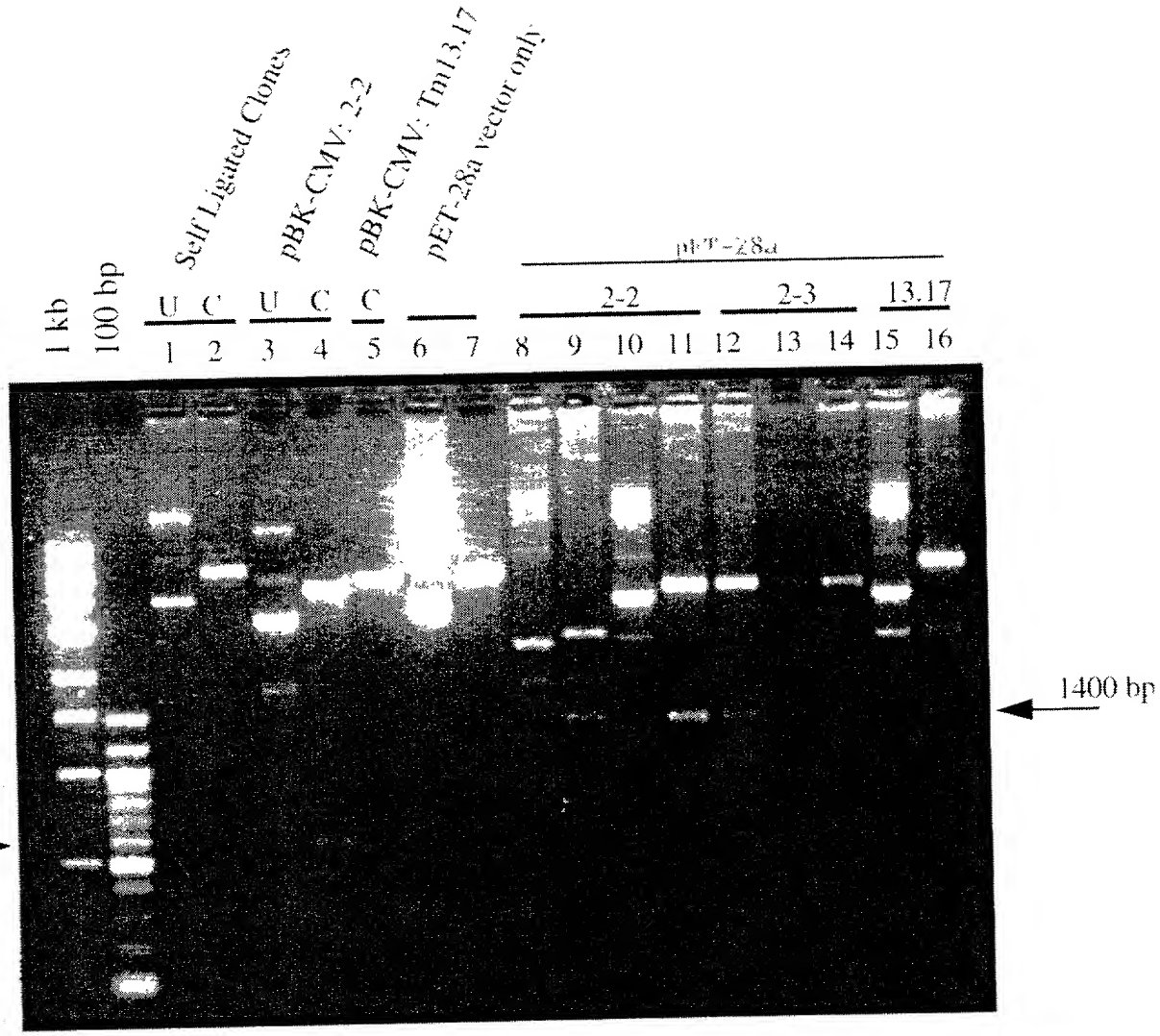


FIG. 5.2

Electronic Acknowledgement Receipt
EFS ID: 6656
Application Number: 09/999,999
International Application Number:
Confirmation Number: 9999
Title of Invention: Test Application
First Named Inventor/Applicant Name: Test Examiner
Customer Number: 99999
Filer: Test Examiner
Filer Authorized By:
Attorney Docket Number: 99999-999999
Receipt Date: 01-JAN-2001
Filing Type: NEW
Time Stamp: 00:00:00
Application Type: Utility
Payment information:
Payment Type: Deposit Account
Payment was successfully received in RAM
RAM confirmation number: 999
Deposit Account: 999999
Authorized User:
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:
Charge any Additional Fee required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)
Charge any Additional Fee required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)
Charge any Additional Fee required under 37 C.F.R. Section 1.19 (Document supply fees)
Charge any Additional Fee required under 37 C.F.R. Section 1.20 (Post Issuance fees)
Charge any Additional Fee required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)
File Listing:
Document Number: 1
Multi Part /.zip: no
Pages (if appl.): 1
Document Description: Test Application
File Size(Bytes): 999999
Error Code:
Error Message:
Warnings:
Information:
Total Files Size (in bytes): 999999
New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is made in compliance with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.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100 bp
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pBK-CMV: Tm13.17
pET-28a vector only
pET-28a
U C U C C
2-2
2-3
13.17
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
650 bp
1400 bp

FIG. 5.3

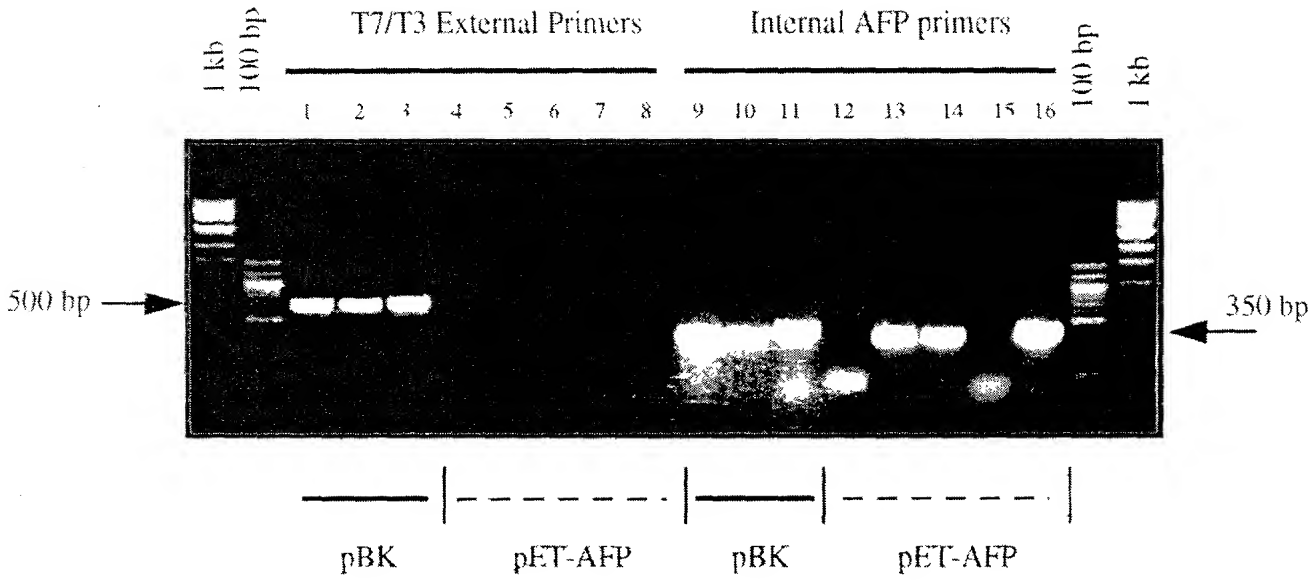

FIG. 5.4

US 6,696,569 B2

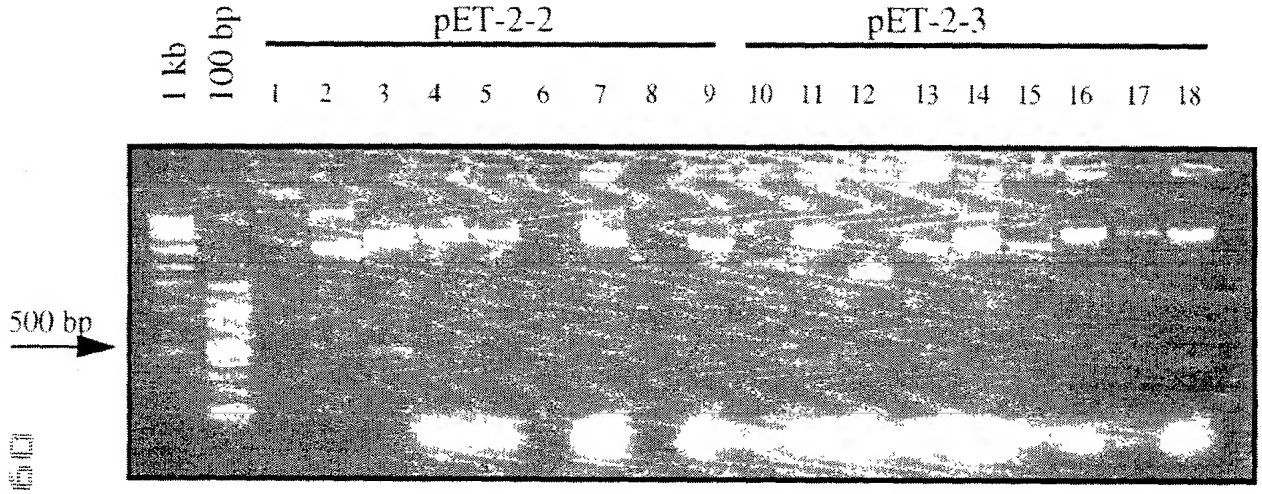

FIG. 5.5

Patent Application Publication Sep. 25, 2008 Sheet 5 of 5 US 2008/0233650

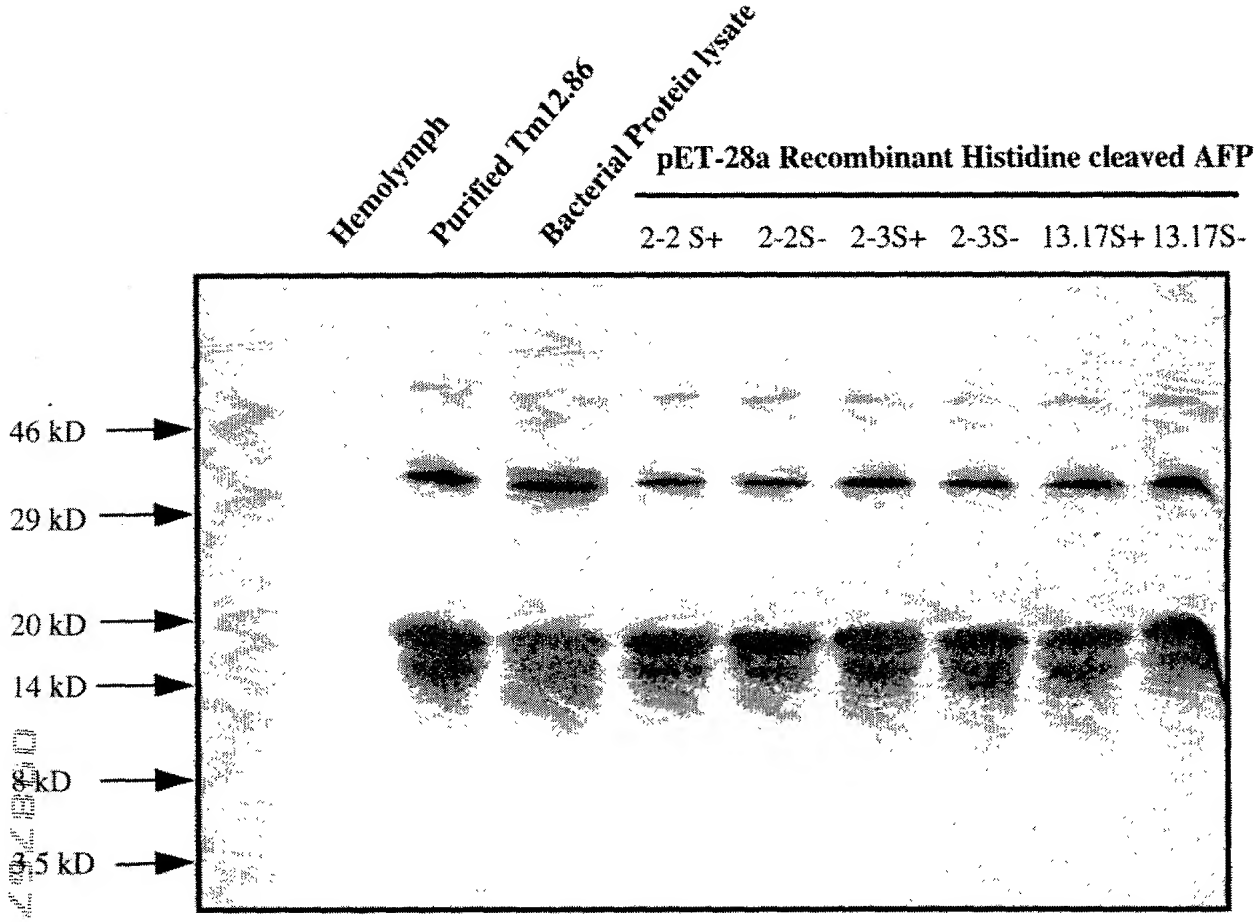

FIG. 5.6

Patent Application Publication

His-tagged Clone 2.2 with signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                  50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC               96
               Met Gly Ser Ser His His His His His His Ser
                           -55                 -50

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT             141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
        -45                 -40                 -35

                                                        AFP Start Codon
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG             186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met
        -30                 -25                 -20

AAA CTC CTC TTG TGC TTT GCG TTC GCC GCC ATC GTC ATC GGA GCT             231
Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala
        -15                 -10                 -5

        N-terminal of mature AFP
CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC             276
Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser
        1               5                   10

AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC             321
Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp
    15                  20                  25

AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG             366
Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys
    30                  35                  40

CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC             411
His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala
    45                  50                  55

GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG             456
Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val
    60                  65                  70

GCC AGC GAC GAA GAG GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC             501
Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val
    75                  80                  85

AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT             546
Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys
    90                  95                  100

                                            Stop Codon
ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA          595
Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp *
    105                 110                 115

                    Polyadenylation signal    Poly-A tail
TTTGACTGAA TTTTGACAAT AAAGGTAATA TCGTTATGTA AAAAAAAAAA                  645

AAAAAACTCG AGCACCACCA CCACCACCAC TGAGAT                                 681
```

FIG. 5.7

His-tagged clone 2.2 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG          50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC      96
               Met Gly Ser Ser His His His His His His Ser
                                -30                 -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT   141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
            -20                 -15                 -10

                                    N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGA TCC CTC ACC GAC GAA CAG ATA CAG   186
Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln
            -5                  1                   5

AAA AGG AAC AAG ATC AGC AAA GAA TGC CAG CAG GTG TCC GGA GTG   231
Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val
        10                  15                  20

TCC CAA GAG ACG ATC GAC AAA GTC CGC ACA GGT GTC TTG GTC GAT   276
Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp
        25                  30                  35

GAT CCC AAA ATG AAG AAG CAC GTC CTC TGC TTC TCG AAG AAA ACT   321
Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr
        40                  45                  50

GGA GTG GCA ACC GAA GCC GGA GAC ACC AAT GTG GAG GTA CTC AAA   366
Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys
        55                  60                  65

GCC AAG CTG AAG CAT GTG GCC AGC GAC GAA GAG GTG GAC AAG ATC   411
Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile
        70                  75                  80

GTG CAG AAG TGC GTG GTC AAG AAG GCC ACA CCA GAG GAA ACG GCT   456
Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala
        85                  90                  95

TAT GAC ACC TTC AAG TGT ATT TAC GAC AGT AAA CCT GAT TTC TCT   501
Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser
        100                 105                 110

            Stop Codon
CCT ATT GAT TAA CTCGAGCACC ACCACCACCA CCACTGAGAT              543
Pro Ile Asp *
        115
```

FIG. 5.8

His-tagged clone 2.3 with signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                  50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC               96
               Met Gly Ser Ser His His His His His His Ser
                          -55                 -50

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT             141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
        -45                 -40                 -35

                                                      AFP Start Codon
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG             186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met
        -30                 -25                 -20

AAA CTC CTC TTG TGC TTT GCT TTC GCC GCC ATC GTC ATC GGA GCT             231
Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala
        -15                 -10                 -5

        N-terminal of Mature AFP
CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC             276
Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser
        1               5                   10

AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC             321
Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp
    15                  20                  25

AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG             366
Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys
    30                  35                  40

CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC             411
His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala
    45                  50                  55

GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG             456
Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val
    60                  65                  70

GCC AGC GAC GAA GAA GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC             501
Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val
    75                  80                  85

AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT             546
Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys
    90                  95                  100

                                                  Stop Codon
ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA          595
Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp *
    105                 110                 115

                    Polyadenylation signal    Poly-A tail
TTTGACTGAA TTTTGACAAT AAAGGTACTA TCGTTATGAA AAAAAAAAAA                  645

AAAAAAACTC GAGCACCACC ACCACCACCA CTGAGAT                                682
```

FIG. 5.9

His-tagged Clone 2.3 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                    50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC                 96
               Met Gly Ser Ser His His His His His His Ser
                              -30                 -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT               141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
          -20                 -15                 -10

                                    N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGA TCC CTC ACC GAC GAA CAG ATA CAG               186
Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln
          -5                  1                   5

AAA AGG AAC AAG ATC AGC AAA GAA TGC CAG CAG GTG TCC GGA GTG               231
Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val
       10                  15                  20

TCC CAA GAG ACG ATC GAC AAA GTC CGC ACA GGT GTC TTG GTC GAT               276
Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp
       25                  30                  35

GAT CCC AAA ATG AAG AAG CAC GTC CTC TGC TTC TCG AAG AAA ACT               321
Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr
       40                  45                  50

GGA GTG GCA ACC GAA GCC GGA GAC ACC AAT GTG GAG GTA CTC AAA               366
Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys
       55                  60                  65

GCC AAG CTG AAG CAT GTG GCC AGC GAC GAA GAA GTG GAC AAG ATC               411
Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile
       70                  75                  80

GTG CAG AAG TGC GTG GTC AAG AAG GCC ACA CCA GAG GAA ACG GCT               456
Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala
       85                  90                  95

TAT GAC ACC TTC AAG TGT ATT TAC GAC AGT AAA CCT GAT TTC TCT               501
Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser
       100                 105                 110

            Stop Codon
CCT ATT GAT TAA CTCGAGCACC ACCACCACCA CCACTGAGAT                          543
Pro Ile Asp *
       115
```

FIG. 5.10

His-tagged Tm 13.17 with signal sequence

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TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                    50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC                 96
               Met Gly Ser Ser His His His His His His Ser
               -65                 -60                 -55

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT               141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
               -50                 -45                 -40

GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC TGG ATC CAA AGA ATT               186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Trp Ile Gln Arg Ile
               -35                 -30                 -25

                        AFP Start Codon
CGG CAC GAG ACT ACT AAG ATG AAG TTG CTC TGT TGT CTA ATC TCC               231
Arg His Glu Thr Thr Lys Met Lys Leu Leu Cys Cys Leu Ile Ser
               -20                 -15                 -10

                                    N-terminal of mature AFP
CTC ATT CTG TTG GTC ACA GTT CAG GCC CTG ACC GAG GCA CAA ATT               276
Leu Ile Leu Leu Val Thr Val Gln Ala Leu Thr Glu Ala Gln Ile
               -5                  1                   5

GAG AAA CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA               321
Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly
           10                  15                  20

GTG TCG CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG               366
Val Ser Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu
           25                  30                  35

GAC GAT CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC               411
Asp Asp Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn
           40                  45                  50

GCC GGT CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG               456
Ala Gly Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu
           55                  60                  65

AGG GAG AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG               501
Arg Glu Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu
           70                  75                  80

AAA ATC ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG               546
Lys Ile Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu
           85                  90                  95

ACG GTG TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG               595
Thr Val Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys
           100                 105                 110

                    Stop Codon
TTC TCA CCA GTT GAT TGA ACCACCACGA CTAGTAGATG GTTCAAATGG                  643
Phe Ser Pro Val Asp *
           115

                  Polyadenylation signal  Poly-A tail
TGTGCTTTAC ATATAAAAAT AAAGTGTTTC TGATGTAAAA AAAAAAAAAA                    693

AAAAAAAAAA AACTCGAGAG TATTCTAGAG CGGCCGCGGG CCCATCGTTT                    743

TCCACCCCTC GAGCACCACC ACCACCACCA CTGAGAT                                  777
```

09351908.030904

FIG. 5.11

His-tagged Tm 13.17 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                  50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC               96
               Met Gly Ser Ser His His His His His His Ser
                           -30                 -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT             141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
        -20                 -15                 -10

                                N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGC CTG ACC GAG GCA CAA ATT GAG AAA             186
Gly Gln Gln Met Gly Arg Gly Leu Thr Glu Ala Gln Ile Glu Lys
        -5                  1                   5

CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA GTG TCG             231
Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly Val Ser
    10                  15                  20

CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG GAC GAT             276
Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu Asp Asp
    25                  30                  35

CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC GCC GGT             321
Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn Ala Gly
    40                  45                  50

CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG AGG GAG             366
Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu Arg Glu
    55                  60                  65

AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG AAA ATC             411
Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu Lys Ile
    70                  75                  80

ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG ACG GTG             456
Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu Thr Val
    85                  90                  95

TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG TTC TCA             501
Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys Phe Ser
    100                 105                 110

            Stop Codon
CCA GTT GAT TGA CTCGAGCACC ACCACCACCA CCACTGAGAT                        543
Pro Val Asp *
    115
```

FIG. 5.12

09690 - 0090 - 0090

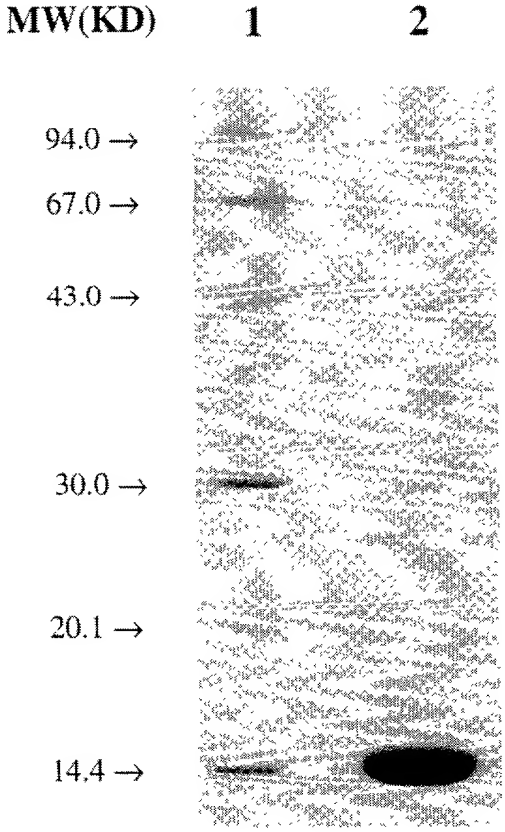

FIG. 6.0

EXPRESS MAIL NO. EV 606 056 525 US

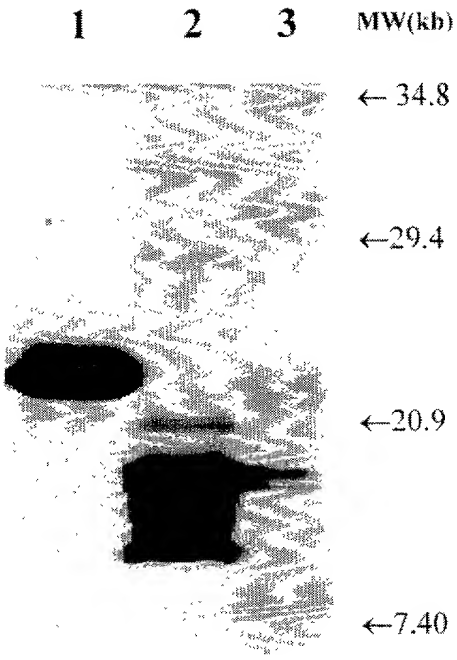

FIG. 6.1

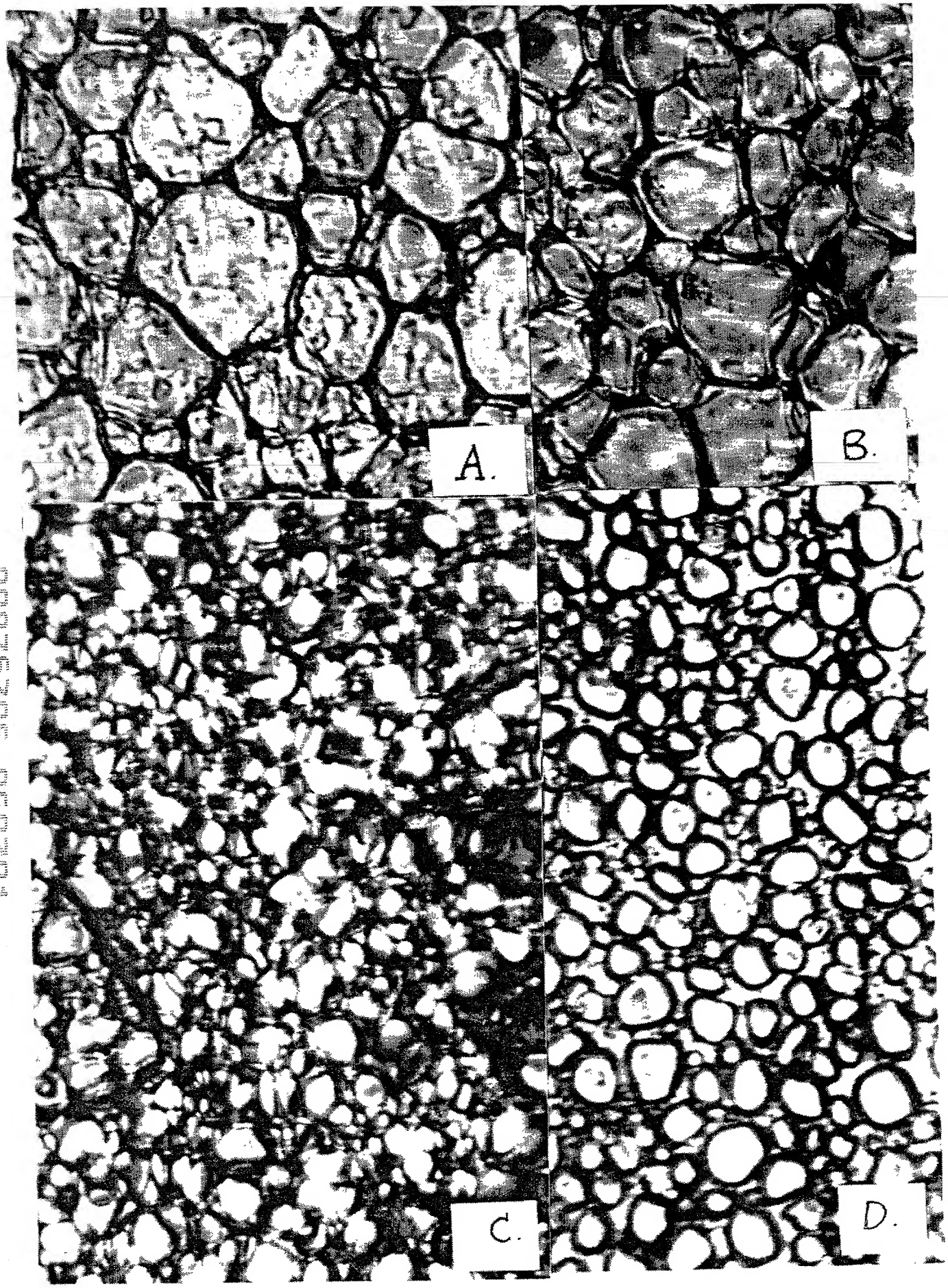

FIG. 6.2

Tm 13.17 S- graph data

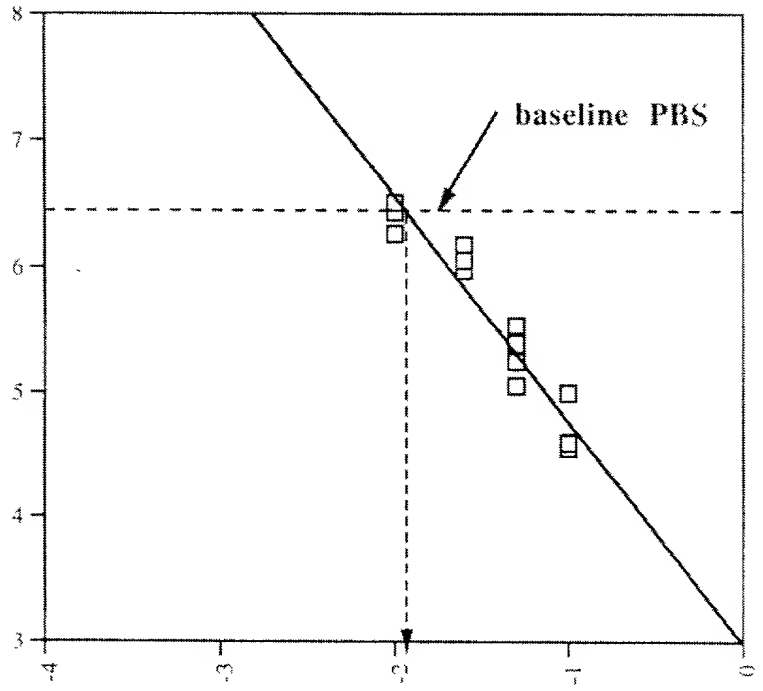

FIG. 6.3

ONE LETTER	NAME	THREE LETTER	CHEMICAL CLASS	HYDROPHOBICITY	Reactivity
A	Alanine	Ala	aliphatic	mod. hydrophobic	low
B	Asp or Asn	Asx			
C	Cysteine	Cys	sulfhydryl	hydrophobic	high
D	Aspartic Acid	Asp	acidic	highly hydrophilic	high
E	Glutamic Acid	Glu	acidic	highly hydrophilic	high
F	Phenylalanine	Phe	aromatic	highly hydrophobic	low
G	Glycine	Gly	aliphatic	mod. hydrophobic	low
H	Histidine	His	basic, imidazole	highly hydrophilic	high
I	Isoleucine	Ile	aliphatic	hydrophobic	low
J					
K	Lysine	Lys	basic	highly hydophilic	high
L	Leucine	Leu	aliphatic	hydrophobic	low
M	Methionine	Met	sulfhydryl	hydrophobic	low
N	Asparagine	Asn	amide, acidic derived	hydrophilic	high
O					
P	Proline	Pro	aliphatic, cyclic, imino	mod. hydrophilic	low
Q	Glutamine	Gln	amide, acidic derived	hydrophilic	high
R	Arginine	Arg	basic	highly hydrophilic	high
S	Serine	Ser	aliphatic hydroxyl	hydrophilic	high
T	Threonine	Thr	aliphatic hydroxyl	hydrophilic	high
U					
V	Valine	Val	aliphatic	hydrophobic	low
W	Tryptophan	Trp	aromatic	highly hydrophobic	low
X					
Y	Tyrosine	Tyr	aromatic	mod. hydrophilic	high
Z	Glu or Gln	Glx			

ACD	Any Acidic
ALP	Any Aliphatic
ALH	Any Aliphatic Hydroxyl
ARO	Any Aromatic
BAS	Any Basic
HY-	Hydrophobic
HY+	Hydrophilic

960960.00004

FIG. 7.1

Position	Tm 12 84-2.2	Tm 12 84-2 3	Tm 12.84-3 4	Tm 12 84-3 9	Tm 12.84-7 5	Concensus of Tm 12 84	Tm13 17	Concensus with Tm 13,17	B1	Concensus with B1	AFP-3	Concensus with AFP-3
1	A	A	A	A	A	A	A	A		A	C	N
2	C	C	C	C	C	C	G	N		N	A	N
3	G	G	G	G	G	G	A	R		R	G	R
4	A	A	A	A	A	A	C	N		N	A	N
5	G	G	G	G	G	G	T	N		N	T	N
6	C	C	C	C	C	C	A	N		N	C	N
7	A	A	A	A	A	A	C	N		N	G	N
8	A	A	A	A	A	A	T	N		N	A	N
9	A	A	A	A	A	A	A	A		A	A	A
10	A	A	A	A	A	A	G	R		R	G	R
11	A	A	A	A	A	A	G	R		R	G	R
12	·	·	·	·	·	·	·	·		·	·	·
13	A	A	A	A	A	A	A	A	A?	A	A	A
14	T	T	T	T	T	T	T	T	T?	T	T	T
15	G	G	G	G	G	G	G	G	G?	G	G	G
16	A	A	A	A	A	A	A	A		A	A	A
17	A	A	A	A	A	A	A	A		A	A	A
18	A	A	A	A	A	A	G	R		R	G	R
19	C	C	C	C	C	C	T	Y		Y	C	Y
20	T	T	T	T	T	T	T	T		T	T	T
21	C	C	C	C	C	C	G	C/G		C/G	C	C/G
22	C	C	C	C	C	C	C	C		C	C	C
23	T	T	T	T	T	T	T	T		T	T	T
24	T	T	T	T	T	T	T	T		T	T	T
25	T	T	T	T	T	T	T	T		T	C	Y
26	T	T	T	T	T	T	T	N		N	C	N
27	G	G	G	G	G	G	T	N		N	T	N
28	T	T	T	T	T	T	T	T		T	G	T
29	G	G	G	G	G	G	G	G		G	T	G
30	C	C	C	C	C	C	C	Y		Y	T	Y
31	T	T	T	T	T	T	C	Y	C	Y	C	Y
32	T	T	T	T	T	T	A	T	T	T	T	T
33	T	T	T	T	T	T	A	T/A	T	T/A	T	T/A
34	G	G	G	G	G	G	A	R	A	R	G	R
35	C	C	C	C	C	C	T	Y	C	Y	T	Y
36	G	G	G	G	G	G	T	N	T	N	C	N
37	T	T	T	T	T	T	C	Y	T	Y	C	Y
38	T	T	T	T	T	T	C	Y	C	Y	T	Y
39	C	C	C	C	C	C	C	Y	T	Y	C	Y
40	G	G	G	G	G	G	C	G/C	C	G/C	G	G/C
41	C	C	C	C	C	C	T	Y	T	Y	T	Y
42	C	C	C	C	C	C	C	Y	C	Y	T	Y
43	G	G	G	G	G	G	A	R	A	R	G	R
44	C	C	C	C	C	C	T	Y	T	Y	C	Y
45	C	C	C	C	C	C	T	Y	T	Y	C	Y
46	A	A	A	A	A	A	C	N	C	N	T	N
47	T	T	T	T	T	T	T	T	T	T	T	T
48	C	C	C	C	C	C	G	C/G	G	C/G	G	C/G
49	G	G	G	G	G	G	T	N	T	N	G	N
50	T	T	T	T	T	T	T	T	T	T	T	T
51	C	C	C	C	C	C	G	C/G	G	C/G	C	C/G
52	A	A	A	A	A	A	G	R	G	R	G	R
53	T	T	T	T	T	T	T	T	T	T	C	Y
54	C	C	C	C	C	C	C	C	C	C	C	C
55	G	G	G	G	G	G	A	R	C	N	C	N
56	G	G	G	G	G	G	C	G/C	C	G/C	C	G/C
57	A	A	A	A	A	A	A	A	A	A	G	R
58	G	G	G	G	G	G	G	G	G	G	A	R
59	C	C	C	C	C	C	T	Y	T	Y	C	Y
60	T	T	T	T	T	T	T	T	T	T	T	T
61	C	C	C	C	C	C	C	C	C	C	C	Y
62	A	A	A	A	A	A	A	A	A	A	T	A
63	G	G	G	G	G	G	G	G	G	G	A	N
64	G	G	G	G	G	G	G	G	G	G	G	G
65	C	C	C	C	C	C	C	C	C	C	C	C
66	T	T	T	T	T	T	C	Y	C	Y	C	Y
67	C	C	C	C	C	C	C	C	A	N		N
68	T	T	T	T	T	T	T	T	T	T		T
69	C	C	C	C	C	C	G	C/G	A	N		N
70	A	A	A	A	A	A	A	A	A	A		A
71	C	C	C	C	C	C	C	C	C	C		C
72	C	C	C	C	C	C	C	C	T	Y		Y
73	G	G	G	G	G	G	G	G	C	G/C	G	G/C
74	A	A	A	A	A	A	A	A	A	A	A	A
75	C	C	C	C	C	C	G	A	G	N	A	N
76	G	G	G	G	G	G	G	G	G	G	A	R
77	A	A	A	A	A	A	A	A	A	N	A	N
78	A	A	A	A	A	A	C	A	A	A	C	A
79	C	C	C	C	C	C	A	C	G	C/G	C	C/G
80	A	A	A	A	A	A	C	A	A	A	C	N
81	G	G	G	G	G	G	A	R	C	N	T	N
82	A	A	A	A	A	A	A	A	T	N	C	N
83	T	T	T	T	T	T	T	T	T	T	G	N
84	A	A	A	A	A	A	T	A/T	T	A/T	T	A/T
85	C	C	C	C	C	C	G	C/G	G	C/G	G	C/G
86	A	A	A	A	A	A	A	A	A	A	A	A
87	G	G	G	G	G	G	A	G	G	G	G	G
88	A	A	A	A	A	A	A	A	C	N	A	N
89	A	A	A	A	A	A	A	A	T	A/T	A	A/T
90	A	A	A	A	A	A	A	A	A	A	G	R
91	A	A	A	A	A	A	C	N	C	N	C	N
92	G	G	G	G	G	G	T	N	T	N	T	N
93	G	G	G	G	G	G	G	G	G	G	G	G
94	A	A	A	A	A	A	A	A	A	N	A	N
95	A	A	A	A	A	A	C	A	C	N	G	N
96	C	C	C	C	C	C	A	C	C	C	C	Y
97	A	A	A	A	A	A	C	A	C	N	C	N
98	A	A	A	A	A	A	G	A	A	A	G	A
99	G	G	G	G	G	G	A	R	A	R	C	R
100	A	A	A	A	A	A	T	A	A	A	G	A
101	T	T	T	T	T	T	C	Y	C	Y	C	Y
102	C	C	C	C	C	C	A	C	C	C	A	C
103	A	A	A	A	A	A	G	A	A	A	G	A
104	G	G	G	G	G	G	C	G	G	G	C	G
105	C	C	C	C	C	C	A	C	G	C	G	C
106	A	A	A	A	A	A	A	A	G	R	A	R
107	A	A	A	A	A	A	A	A	C	N	C	N
108	A	A	A	A	A	A	A	A	A	A	G	R
109	A	A	A	A	A	A	A	A	A	A	C	N
110	A	A	A	A	A	A	A	A	A	A	G	R
111	A	A	A	A	A	A	A	A	A	A	C	N
112	T	T	T	T	T	T	T	T	T	T	C	T
113	G	G	G	G	G	G	T	G	T	G	T	G
114	C	C	C	C	C	C	G	Y	G	Y	G	Y
115	C	C	C	C	C	C	T	C	C	C	C	C
116	A	A	A	A	A	A	C	A	A	A	A	A
117	G	G	G	G	G	G	A	R	G	R	G	R

DRAFT

FIG. 7.2

Position	Tm 12.84-2 2	Tm 12 84-2.3	Tm 12 84-3 4	Tm 12 84-3 9	Tm 12 84-7 5	Concensus of	Tm13 17	Concensus with	B1	Concensus with	AFP-3	Concensus with
118	C	C	C	C	C	C	A	N	A	N	G	N
119	A	A	A	A	A	A	A	N	C	N	C	N
120	G	G	G	G	G	G	T	N	T	N	T	N
121	G	G	G	G	G	G	G	G	G	G	G	G
122	T	T	T	A	A	T/A	A	T/A	A	T/A	A	A/T
123	T	T	T	G	G	G	A	R	A	R	G	R
124	T	T	T	T	T	T/A	A	T/A	A	T/A	T	T/A
125	C	C	C	C	C	C/G	G	C/G	T	C/G	C	C/G
126	C	C	C	C	C	C	T	Y	G	Y	T	Y
127	G	G	G	G	G	G	G	G	G	G	G	G
128	G	G	G	G	G	G	A	G	G	G	G	G
129	A	A	A	A	A	A	G	A	A	A	A	A
130	G	G	G	G	G	G	T	G	G	G	G	G
131	T	T	T	T	T	T	G	T	T	T	A	T
132	G	G	G	G	G	G	T	G	G	G	T	R
133	T	T	T	T	T	T	C	T	T	T	C	T
134	C	C	C	C	C	C	G	C	C	C	T	C
135	C	C	C	C	C	C	C	C/G	C	C/G	C	N
136	C	C	C	C	C	C	A	C/G	A	C/G	T	C/G
137	A	A	A	A	A	A	A	A	A	A	G	A
138	A	A	A	A	A	A	G	A	A	A	A	A
139	A	A	A	A	A	A	A	A	A	A	G	A
140	A	A	A	A	A	A	G	A	A	A	A	A
141	A	A	A	A	A	A	A	G/C	G	G/C	G	G/C
142	A	A	A	A	A	A	T	R	T	N	C	N
143	C	C	C	C	C	C	C	Y	C	Y	C	Y
144	G	G	G	G	G	G	A	G/C	C	G/C	C	G/C
145	A	A	A	A	A	A	T	A	T	N	T	N
146	T	T	T	T	T	T	A	N	A	R	C	R
147	C	C	C	C	C	C	T	R	A	N	T	N
148	G	G	G	G	G	G	C	N	A	C/G	C	C/G
149	A	A	A	A	A	A	A	C	A	A	A	A
150	C	C	C	C	C	C	A	A	A	R	A	R
151	A	A	A	A	A	A	A	A	A	A	G	R
152	A	A	A	A	A	A	A	A	A	A	T	G
153	A	A	A	A	A	A	G	A	A	Y	T	Y
154	A	A	A	A	A	A	T	Y	G	Y	C	Y
155	G	G	G	G	G	G	C	Y	C	Y	G	C
156	T	T	T	T	T	T	T	C	T	C	C	C
157	C	C	C	C	C	C	C	G	C	G	G	G
158	C	C	C	C	C	C	G	C	C	C	C	C
159	G	G	G	G	G	G	C	C	C	C	A	C
160	C	C	C	C	C	C	A	A	A	A	A	A
161	A	A	A	A	A	A	A	N	A	N	A	N
162	C	C	C	C	C	C	A	G	A	G	C	G/C
163	A	A	A	A	A	A	C	G	A	G	G	G
164	G	G	G	G	G	G	G	T	G	T	C	T
165	G	G	G	G	G	G	T	G	T	G	G	G
166	T	T	T	T	T	T	G	T/A	G	T/A	G	T/A
167	C	C	C	C	C	C	A	C	A	C	A	A
168	T	T	T	T	T	T	C	T	C	T	A	N
169	T	T	T	T	T	T	T	N	T	N	A	N
170	T	T	T	T	T	T	G	G	G	G	A	R
171	G	G	G	G	G	G	G	T/A	G	T/A	G	T/A
172	T	T	T	T	T	T	G	C/G	G	C/G	T	C/G
173	C	C	C	C	C	C	A	G	A	G	G	G
174	G	G	G	G	G	G	G	A	G	A	G	A
175	A	A	A	A	A	A	G	Y	G	Y	G	Y
176	T	T	T	T	T	T	A	G	A	G	A	G
177	G	G	G	G	G	G	T	A	T	A	C	A
178	A	A	A	A	A	A	C	T	C	T	G	T
179	T	T	T	T	T	T	C	C	C	C	A	C
180	C	C	C	C	C	C	T	Y	C	Y	C	Y
181	C	C	C	C	C	C	A	A	A	A	C	A
182	C	C	C	C	C	C	A	A	A	A	C	A
183	C	C	C	C	C	C	A	N	A	N	T	N
184	A	A	A	A	A	A	A	T	A	T	G	T
185	A	A	A	A	A	A	A	G	A	G	A	G
186	A	A	A	A	A	A	A	A	A	A	A	A
187	A	A	A	A	A	A	A	R	A	R	R	R
188	T	T	T	T	T	T	C	N	A	N	N	N
189	G	G	G	G	G	G	T	G/C	C	G/C	A	G/C
190	A	A	A	A	A	A	T	C	A	C	G	C
191	A	A	A	A	A	A	T	A	A	A	C	A
192	G	G	G	G	G	G	T	N	A	N	A	N
193	A	A	A	A	A	A	T	G/C	A	G/C	T	G/C
194	A	A	A	A	A	A	T	T	A	T	G	T
195	A	A	A	A	A	A	T	Y	A	Y	C	Y
196	G	G	G	G	G	G	C	T	A	T	A	T
197	C	C	C	C	C	C	A	Y	A	Y	T	Y
198	A	A	A	A	A	A	C	T	A	T	T	T
199	C	C	C	C	C	C	G	T	A	T	T	T
200	G	G	G	G	G	G	T	T	A	T	T	T
201	T	T	T	T	T	T	T	T	A	T	T	T
202	C	C	C	C	C	C	T	T	A	T	T	T
203	C	C	C	C	C	C	T	T	A	T	T	T
204	T	T	T	T	T	T	T	T	A	T	T	T
205	C	C	C	C	C	C	T	T	A	T	C	T
206	T	T	T	T	T	T	G	T	A	T	T	T
207	G	G	G	G	G	G	C	G	A	G	T	G
208	C	C	C	C	C	C	T	T	A	T	T	T
209	T	T	T	T	T	T	T	T	A	T	T	T
210	T	T	T	T	T	T	T	T	A	T	T	T
211	C	C	C	C	C	C	T	T	A	T	T	T
212	T	T	T	T	T	T	G	T	A	T	T	T
213	C	C	C	C	C	C	C	G	A	G	T	G
214	G	G	G	G	G	G	C	C	A	C	C	C
215	A	A	A	A	A	A	A	A	A	A	A	A
216	A	A	A	A	A	A	G	G	A	G	T	G
217	A	A	A	A	A	A	A	A	A	A	C	A
218	A	A	A	A	A	A	A	A	A	A	T	A
219	A	A	A	A	A	A	A	A	A	A	C	A
220	A	A	A	A	A	A	A	A	A	A	T	A
221	C	C	C	C	C	C	A	A	A	A	C	A
222	T	T	T	T	T	T	A	A	A	A	C	A
223	G	G	G	G	G	G	A	A	A	A	C	A
224	A	A	A	A	A	A	A	A	A	A	C	A
225	G	G	G	G	G	G	A	A	A	A	C	A
226	T	T	T	T	T	T	G	A	A	A	C	A
227	G	G	G	G	G	G	T	G	A	G	C	G
228	C	C	C	C	C	C	G	G	A	G	C	G
229	A	A	A	A	A	A	G	C	A	C	C	C
230	A	A	A	A	A	A	C	A	A	A	C	A
231	C	C	C	C	C	C	A	A	A	A	C	A
232	C	C	C	C	C	C	C	A	A	A	C	A
233	C	C	C	C	C	C	G	C	A	C	C	C
234	G	G	G	G	G	G	G	C	A	C	C	C
235	G	G	G	G	G	G	G	C/G	G	C/G	G	C/G

FIG. 7.2 Cont.

Position	Tm 12.84-2 2	Tm 12.84-2 3	Tm 12.84-3 4	Tm 12.84-3 9	Tm 12.84-7 5	Concensus of	Tm13 17	Concensus with	B1	Concensus with	AFP 3	Concensus with
236	A	A	A	A	A	A	A	A	A	A	C	N
237	A	A	A	A	A	A	A	A	A	A	C	N
238	G	G	G	G	G	G	T	N	T	N	A	N
239	C	C	C	C	C	C	C	C	G	C	G	C/G
240	C	C	C	C	C	C	G	C/G	G	C/G	T	N
241	G	G	G	G	G	G	G	G	G	G	G	G
242	G	G	G	G	G	G	G	G	G	G	G	G
243	A	A	A	A	A	A	A	A	A	A	T	A/T
244	G	A	G	A	G	A	G	A	G	A	G	A
245	A	A	A	A	A	A	A	A	A	A	A	A
246	C	A	C	A	C	A	G	C/G	A	A	A	T
247	A	A	A	A	A	A	G	R	A	R	T	N
248	C	C	C	C	C	C	T	Y	T	Y	T	Y
249	C	C	C	C	C	C	G	C/G	T	N	C	N
250	A	A	A	A	A	A	G	R	G	R	C	N
251	A	A	A	A	A	A	T	A/T	A	A/T	A	A/T
252	T	G	T	G	T	G	T	N	G	N	A	N
253	G	T	G	T	G	T	G	G	G	G	C	G/C
254	T	G	T	G	T	G	T	T	C	Y	T	Y
255	G	G	G	G	G	G	C	G/C	C	G/C	G	G/C
256	G	G	G	G	G	G	G	G	G	G	G	G
257	A	A	A	A	A	A	A	A	A	A	G	A
258	G	G	G	G	G	G	C	G/C	A	G/C	C	G/C
259	G	T	G	T	G	T	G	G	A	R	C	N
260	T	A	T	A	T	A	T	T	C	Y	A	N
261	A	C	A	C	A	C	T	R	G	R	C	N
262	C	C	C	C	C	C	T	Y	T	Y	A	N
263	T	C	T	C	T	C	T	T	T	T	T	N
264	C	A	C	A	C	A	G	C/G	C	C/G	T	N
265	A	A	A	A	A	A	A	A	A	A	A	A
266	A	A	A	A	A	A	G	R	A	R	A	R
267	A	G	A	G	A	G	G	R	G	R	G	R
268	G	C	G	C	G	C	G	G	G	G	A	R
269	C	C	C	C	C	C	A	N	A	N	C	N
270	C	A	C	A	C	A	G	C/G	G	C/G	G	C/G
271	A	A	A	A	A	A	A	A	A	A	A	A
272	A	G	A	G	A	G	A	A	A	A	A	A
273	G	C	G	C	G	C	G	G	G	G	A	R
274	C	T	C	T	C	T	G	C/G	T	N	T	N
275	T	G	T	G	T	G	T	T	T	T	T	T
276	G	A	G	A	G	A	G	G	G	G	C	G/C
277	A	A	A	A	A	A	A	A	A	A	A	A
278	A	G	A	G	A	G	A	R	C	N	A	N
279	G	C	G	C	G	C	G	G	G	G	G	G
280	C	A	C	A	C	A	A	N	A	N	G	N
281	A	T	A	T	A	T	A	A	G	R	G	R
282	T	G	T	G	T	G	G	N	G	N	A	N
283	G	T	G	T	G	T	G	G	T	N	A	N
284	T	G	T	G	T	G	T	T	G	N	A	N
285	G	G	G	G	G	G	C	G/C	A	N	C	N
286	G	C	G	C	G	C	A	R	C	N	T	N
287	C	C	C	C	C	C	C	C	A	N	C	N
288	C	A	C	A	C	A	C	Y	A	N	T	N
289	A	G	A	G	A	G	A	R	A	R	G	R
290	G	C	G	C	G	C	A	R	C	N	A	N
291	C		C		C		C	C	G	C/G	G	C/G
292							A	A	G	A	G	R
293							A	A	T	A/T	G	A/T
294							C	C/G	G	C/G	A	N
295	G	G	G	G	G	G	G	G	A	R	T	N
296	A	A	A	A	A	A	A	A	T	A/T	C	N
297	C	C	C	C	C	C	C	C	G	C/G	G	C/G
298	G	G	G	G	G	G	G	G	A	R	G	R
299	A	A	A	A	A	A	A	A	A	A	A	A
300	A	A	A	A	A	A	A	A	G	A	G	A
301	G	G	G	G	G	G	G	G	A	R	A	R
302	A	A	A	A	A	A	A	A	A	A	G	A
303	G	A	G	A	G	A	R	R	A	R	A	R
304	G	T	G	T	G	T	A	R	G	R	T	R
305	T	G	T	G	T	G	A	R	C	Y	C	Y
306	G	T	G	T	G	T	C	T	G	N	G	N
307	G	A	G	A	G	A	T	G	A	R	T	R
308	A	C	A	C	A	C	G	A	G	R	C	R
309	C	A	C	A	C	A	A	A	A	N	A	N
310	A	A	A	A	A	A	A	A	G	A	T	R
311	A	G	A	G	A	G	A	A	A	R	G	R
312	G	A	G	A	G	A	A	A	T	R	A	N
313	A	T	A	T	A	T	T	A	T	A/T	C	A/T
314	T	C	T	C	T	C	C	T	T	T	T	T
315	C	G	C	G	C	G	G	C/G	T	C/G	T	C/G
316	G	T	G	T	G	T	T	R	C	N	G	N
317	T	G	T	G	T	G	C	T	T	Y	T	Y
318	G	C	G	C	G	C	A	G/C	G	G/C	T	N
319	C	A	C	A	C	A	A	N	A	N	T	N
320	A	G	A	G	A	G	A	A	G	R	G	N
321	G	A	G	A	G	A	T	N	A	N	C	N
322	A	A	A	A	A	A	A	A	A	A	A	A
323	A	G	A	G	A	G	A	A	G	R	A	R
324	G	A	G	A	G	A	A	G	T	N	A	N
325	T	G	T	G	T	G	T	T	G	N	A	N
326	G	C	G	C	G	C	T	G	C	G/C	T	G/C
327	C	G	C	G	C	G	G	C	A	N	G	N
328	G	T	G	T	G	T	C	G	C	G/C	C	G/C
329	T	G	T	G	T	G	G	Y	G	N	C	N
330	G	T	G	T	G	T	C	G	G	G/C	C	G/C
331	G	T	G	T	G	T	G	G	T	N	G	N
332	T	C	T	C	T	C	T	T	G	N	T	N
333	C	A	C	A	C	A	C	A	A	N	C	N
334	A	A	A	A	A	A	A	A	C	N	A	N
335	A	G	A	G	A	G	A	A	T	N	A	N
336	G	A	G	A	G	A	G	G	G	A	G	G
337	A	A	A	A	A	A	A	A	A	R	A	A
338	A	G	A	G	A	G	A	A	C	N	A	R
339	G	C	G	C	G	C	G	G	A	R	G	N
340	C	C	C	C	C	C	A	N	C	N	A	R
341	C	A	C	A	C	A	T	Y	A	N	C	N
342	A	C	A	C	A	C	A	A	C	N	A	N
343	C	A	C	A	C	A	C	C	T	Y	C	Y
344	A	C	A	C	A	C	A	A	T	N	T	N
345	C	A	C	A	C	A	C	C	G	C/G	C	C/G
346	C	C	C	C	C	C	T	T	C	Y	C	T
347	C	A	C	A	C	A	G	G	G	N	T	N
348	A	G	A	G	A	G	T	A/T	A	R	C	N
349	G	A	G	A	G	A	G	G	A	A	T	N
350	A	G	A	G	A	G	A	A	G	R	C	A
351	G	G	G	G	G	G	A	R	A	R	A	R
352	G	G	G	G	G	G	G	G	T	A/T	C	A/T
353	A	A	A	A	A	A	A	A	A	A	A	A

FIG. 7.2 Cont.

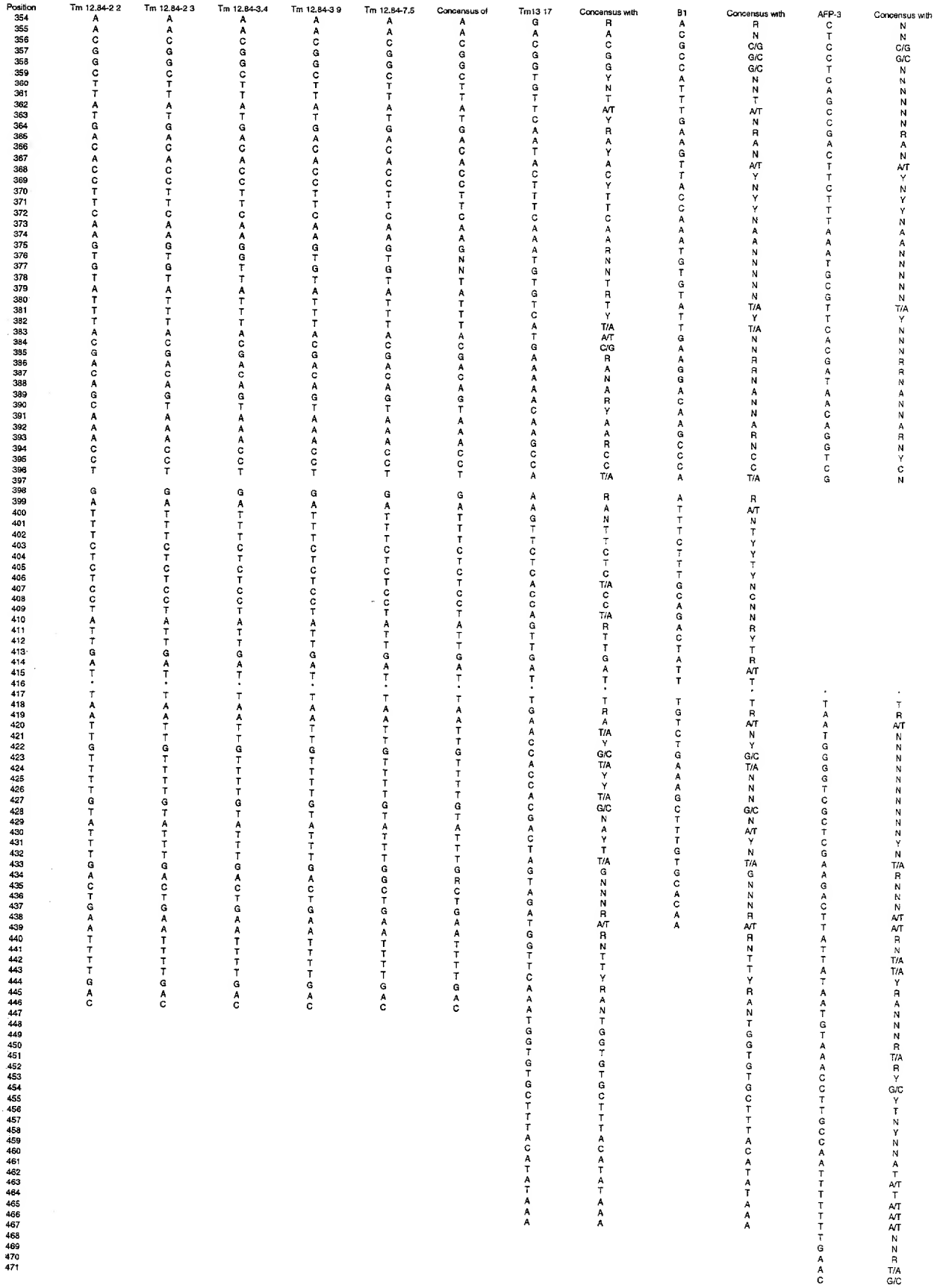
Position
Tm 12.84-2 2
Tm 12.84-2 3
Tm 12.84-3.4
Tm 12.84-3 9
Tm 12.84-7.5
Concensus of
Tm13 17
Concensus with
B1
Concensus with
AFP-3
Concensus with

FIG. 7.2 Cont.

Position	Tm 12 84-2 2	Tm 12 84-2 3	Tm 12 84-3 4	Tm 12 84-3.9	Tm 12.84-7 5	Concensus of	Tm13.17	Concensus with	B1	Concensus with	AFP-3	Concensus with
472	A	A	A	A	A	A	A	A		A	A	A
473	A	A	A	A	A	A	A	A		A	A	A
474	T	T	T	T	T	T	T	T		T	T	T
475	A	A	A	A	A	A	A	A		A	A	A
476	A	A	A	A	A	A	A	A		A	A	A
477	A	A	A	A	A	A	A	A		A	A	A
478	G	G	G	G	G	G	G	G		G	T	N
479	G	G	G	G	G	G	T	N		N	T	N
480	T	T	T	T	T	T	G	N		N	T	N
481	A	A	A	A	A	A	T	N		N	A	N
482	A	C	C	C	C	C	T	Y		Y	A	N
483	T	T	T	T	T	T	T	T		T	A	A/T
484	A	A	A	A	A	A	C	N		N	G	N
485	T	T	T	T	T	T	T	N		T	A	A/T
486	C	C	C	C	C	C	G	N		N	A	N
487	G	G	G	G	G	G	A	R		R	A	R
488	T	T	T	T	T	T	T	T		T	A	T/A
489	T	T	T	T	T	T	G	N		N	A	N
490	A	A	A	A	A	A	T	N		N	A	N
491	T	T	T	T	T	T	A	N		N	A	R
492	G	G	G	G	G	G	A	R		R	A	N
493	T	A	T	A	T	N	A	N		N	A	N
494	A	A	A	A	A	A	A	A		A	A	A
495	A	A	A	A	A	A	A	A		A	A	A
496	A	A	A	A	A	A	A	A		A	A	A
497	A	A	A	A	A	A	A	A		A	A	A
498	A	A	A	A	A	A	A	A		A	A	A
499	A	A	A	A	A	A	A	A		A	A	A
500	A	A	A	A	A	A	A	A		A	A	A
501	A	A	A	A	A	A	A	A		A	A	A
502	A	A	A	A	A	A	A	A		A	A	A
503	A	A	A	A	A	A	A	A		A	A	A
504	A	A	A	A	A	A	A	A		A	A	A
505	A	A	A	A	A	A	A	A		A	A	A
506	A	A	A	A	A	A	A	A		A	A	A
507	A	A	A	A	A	A	A	A		A	A	A
508	A	A	A	A	A	A	A	A		A	A	A
509	A	A	A	A	A	A	A	A		A	A	A
510	A	A	A	A	A	A	A	A		A	A	A
511	A	A	A	A	A	A	A	A		A	A	A
512												

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FIG. 7.2 Cont.

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Position	Tm 12.84-2.2	Tm 12.84-2.3	Tm 12.84-3,4	Tm 12.84-3.9	Tm 12.84-7.5	Concensus Tm 12.84	Tm 13.17	Concensus to Tm13.17	Tm P-B1	Tm P-B2	Concensus to B1/B2	Tm AFP-3	Concensus to AFP-3	GENERAL CONCENSUS	SUBSTITUTIONS - most to least common			
1	M	M	M	M	M	M	M	M			M, OR GAP	M	M, OR GAP	M				
2	K	K	K	K	K	K	K	K			K, OR GAP	K	K, OR GAP	K				
3	L	L	L	L	L	L	L	L			L, OR GAP	L	L, OR GAP	L				
4	L	L	L	L	L	L	L	L			L, OR GAP	L	L, OR GAP	L				
5	L	L	L	L	L	L	C	HY-, ALP/SUL			HY-, ALP/SUL, OR GAP	L	HY-, ALP/SUL, OR GAP	L	C			
6	C	C	C	C	C	C	C	C			C OR GAP	C	C OR GAP	C				
7	F	F	F	F	F	F	L	HY- ARO/ALP	L	L	HY- ARO/ALP	L	HY-, ARO/ALP	F	L			
8	A	A	A	A	A	A	I	ALP	T	T	ALP/ALH	V	ALP/ALH	A	I	T	V	
9	F	F	F	F	F	F	S	ARO/ALH	S	S	ARO/ALH	L	ARO/ALH/ALP	F	S	L		
10	A	A	A	A	A	A	L	ALP	L	L	ALP	V	ALP	A	L	V		
11	A	A	A	A	A	A	I	ALP	I	I	ALP	A	ALP	A	I			
12	I	I	I	I	I	I	L	ALP	L	L	ALP	L	ALP	I	L			
13	V	V	V	V	V	V	L	ALP	L	L	ALP	V	ALP	V	L			
14	I	I	I	I	I	I	V	ALP	V	V	ALP	A	ALP	I	V	A		
15	G	G	G	G	G	G	T	ALP/ALH	A	A	ALP/ALH	A	ALP/ALH	G	T	A		
16	A	A	A	A	A	A	V	ALP	V	V	ALP	T	ALP/ALH	A	V	T		
17	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Y	ACD/ARO	Q	Y			
18	A	A	A	A	A	A	A	A	A	A	A	A	A	A				
19	L	L	L	L	L	L	L	L	I	L	ALP		ALP OR GAP	L	I			
20	T	T	T	T	T	T	T	T	T	T	T		T OR GAP	T				
21	D	D	D	D	D	D	E	ACD	E	E	ACD	E	ACD	D	E			
22	E	E	E	E	E	E	A	ACD/ALP	E	E	ACD/ALP	T	ACD/ALP/ALH	E	A	T		
23	Q	Q	Q	Q	Q	Q	Q	Q	D	D	ACD	P	ACD/ALP	Q	D	P		
24	I	I	I	I	I	I	I	I	L	L	ALP	R	ALP/BAS	I	L	R		
25	Q	Q	Q	Q	Q	Q	E	GLX	E	Q	GLX	E	GLX	Q	E			
26	K	K	K	K	K	K	K	K	L	L	BAS/ALP	K	BAS/ALP	K	L			
27	R	R	R	R	R	R	L	BAS/ALP	L	L	BAS/ALP	L	BAS/ALP	R	L			
28	N	N	N	N	N	N	N	N	R	R	HY+ BAS/ACD	K	HY+ BAS/ACD	N	R	K		
29	K	K	K	K	K	K	K	K	Q	Q	HY+, BAS/ACD	Q	HY+ BAS/ACD	K	Q			
30	I	I	I	I	I	I	T	I	T	T	ALP/ALH	H	ALP/ALH/BAS	I	T	H		
31	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
32	K	K	K	K	K	K	K	K	A	A	BAS/ALP	D	BAS/ALP/ACD	K	A	D		
33	E	E	E	E	E	E	K	HY+ ACD/BAS	E	E	HY+, ACD/BAS	A	ACD/BAS/ALP	E	K	A		
34	C	C	C	C	C	C	C	C	C	C	C	C	C	C				
35	Q	Q	Q	Q	Q	Q	Q	Q	K	K	HY+ ACD/BAS	K	HY+ ACD/BAS	Q	K			
36	Q	Q	Q	Q	Q	Q	N	ACD	T	T	HY+, ACD/ALH	A	ACD/ALH/ALP	Q	N	T	A	
37	V	V	V	V	V	ALP/ACD	E	ACD/ALP	E	E	ACD/ALP	E	ACD/ALP	E	V		(3 9, Tm13 17 B1, B2, AFP-3)	
38	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
39	G	G	G	G	G	G	G	G	G	G	G	G	G	G				
40	V	V	V	V	V	V	V	V	A	A	ALP	V	ALP	V	A			
41	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
42	Q	Q	Q	Q	Q	Q	Q	Q	E	E	GLX	E	GLX	Q	E			
43	E	E	E	E	E	E	E	E	D	A	ACD/ALP	E	ACD/ALP	E	D	A		
44	T	T	T	T	T	T	I	ALP	V	V	ALP	S	ALP/ALH	T	I	V	S	
45	I	I	I	I	I	I	I	I	I	I	I	L	ALP	I	L			
46	D	D	D	D	D	D	T	HY+ ACD/ALH	K	K	HY+, ACD/ALH/BAS	N	HY+ ACD/ALH/BAS	D	T	K	N	
47	K	K	K	K	K	K	K	K	R	K	BAS	K	BAS	K	R			
48	V	V	V	V	V	V	A	ALP	A	A	ALP	V	ALP	V	A			
49	R	R	R	R	R	R	R	R	R	R	R	R	R	R				
50	T	T	T	T	T	T	N	HY+ ALH/ACD	K	K	HY+, ALH/ACD/BAS	N	HY+, ALH/ACD/BAS	T	N	K		
51	G	G	G	G	G	G	G	G	G	G	G	R	ALP/BAS	G	R			
52	V	V	V	V	V	V	D	ALP/ACD	D	D	ALP/ACD	E	ALP/ACD	V	D	E		
53	L	L	L	L	L	L	W	HY- ALP/ARO	L	L	HY- ALP/ARO	E	ALP/ARO/ACD	L	W	E		
54	V	V	V	V	V	V	E	ALP/ACD	E	E	ALP/ACD	V	ALP/ACD	V	E			
55	D	D	D	D	D	D	D	D	D	D	D	D	D	D				
56	D	D	D	D	D	D	D	D	D	D	D	D	D	D				
57	P	P	P	P	P	P	P	P	P	P	P	P	P	P				
58	K	K	K	K	K	K	K	K	K	K	K	K	K	K				
59	M	M	M	M	M	M	L	HY-, SUL/ALP	L	L	HY- SUL/ALP	L	HY- SUL/ALP	M	L			
60	K	K	K	K	K	K	K	K	K	K	K	K	K	K				
61	K	K	K	K	K	K	R	BAS	M	M	BAS/SUL	E	BAS/SUL/ACD	K	R	M	E	
62	H	H	H	H	H	H	Q	HY+, BAS/ACD	Q	Q	HY+, BAS/ACD	H	HY+ BAS/ACD	H	Q			
63	V	V	V	V	V	V	V	V	L	L	ALP	A	ALP	V	L	A		
64	L	L	L	L	L	L	F	HY- ALP/ARO	L	L	HY- ALP/ARO	F	HY- ALP/ARO	L	F			
65	C	C	C	C	C	C	C	C	C	C	C	C	C	C				
66	F	F	F	F	F	F	V	HY-, ARO/ALP	I	I	HY- ARO/ALP	I	HY-, ARO/ALP	F	V	I		
67	S	S	S	S	S	S	A	ALH/ALP	F	F	ALH/ALP/ARO	L	ALH/ALP/ARO	S	A	F	L	
68	K	K	K	K	K	K	R	BAS	K	K	BAS	K	BAS	K	R			
69	K	K	K	K	R	K	BAS	HY+, BAS/ACD	A	A	BAS/ACD/ALP	R	BAS/ACD/ALP	K	R	A	N	(2 2 23 3 4 7 5)
70	T	T	T	T	T	T	A	ALP	L	L	ALP	A	ALP	T	A	L		
71	G	G	G	G	G	G	G	G	I	E	ALP/ACD	G	ALP/ACD	G	I	E		
72	V	V	V	V	V	V	L	ALP	E	I	ALP/ACD	F	ALP/ACD/ARO	V	L	I	E	F
73	A	A	A	A	A	A	A	A	V	V	ALP	I	ALP	A	V	I		
74	T	T	T	T	T	T	T	T	A	A	ALH/ALP	D	ALH/ALP/ACD	T	A	D		
75	E	E	E	E	E	E	E	E	E	E	E	A	ACD/ALP	E	A			
76	A	A	A	A	A	A	S	ALP/ALH	S	S	ALP/ALH	S	ALP/ALH	S	A			
77	G	G	G	G	G	G	G	G	G	G	G	G	G	G				
78	D	D	D	D	D	D	E	ACD	E	E	ACD	E	ACD	E	D			
79	T	T	T	T	T	T	V	ALP	I	I	ALP	F	ALP/ARO	T	I	V	F	
80	N	N	N	N	N	N	V	ACD/ALP	E	E	ACD/ALP	Q	ACD/ALP	N	E	V	Q	
81	V	V	V	V	V	V	V	V	A	A	ALP	L	ALP	V	A	L		
82	E	E	E	E	E	E	D	ACD	D	D	ACD	D	ACD	D	E			
83	V	V	V	V	V	V	V	V	T	T	ALP/ALH	H	ALP/ALH/BAS	V	T	H		
84	L	L	L	L	K	L	L	L	F	F	ALP/ARO	I	ALP/ARO	L	F	I		
85	K	K	K	K	K	K	R	BAS	K	K	BAS	K	BAS	K	R			
86	A	A	A	A	K	A	E	ALP/ACD	E	E	ALP/ACD	T	ALP/ACD/ALH	A	E	T		
87	K	K	K	K	K	K	K	K	K	K	K	K	K	K				
88	L	L	L	L	L	L	L	ALP	L	L	ALP	F	ALP/ARO	L	V	F		
89	K	K	K	K	K	K	R	BAS	T	T	BAS/ALH	K	BAS/ALH	K	T	R		
90	H	H	H	H	H	H	K	BAS	R	R	BAS	E	BAS/ACD	H	R	K	E	
91	V	V	V	V	A	V	V	V	V	V	V	N	ALP/ACD	V	N			
92	A	A	A	A	A	A	T	ALP/ALH	T	T	ALP/ALH	S	ALP/ALH	A	T	S		
93	S	S	S	S	S	S	D	HY+ ALH/ACD	N	N	HY+ ALH/ACD	E	HY+ ALH/ACD	S	N	D	E	
94							N	N OR GAP	D	D	ACD OR GAP	H	ACD OR GAP	D	N	H		
95	D	D	D	D	D	D	D	D	D	D	D	P	ACD/ALP	D	P			
96	E	E	E	E	E	E	E	E	E	E	E	L	E	E				
97	E	E	E	E	F	F	E	E	E	E	E	K	HY+ ACD/BAS	E	K			

FIG. 7.3

TO2090 "96284860

Position	Tm 12.84-2.2	Tm 12.84-2.3	Tm 12.84-3.4	Tm 12.84-3.9	Tm 12.84-7.5	Consensus
98	V	V	V	V	V	V
99	D	D	D	D	D	D
100	K	K	K	K	K	K
101	I	I	I	I	I	I
102	V	V	V	V	V	V
103	Q	Q	Q	Q	Q	Q
104	K	K	K	K	K	K
105	C	C	C	C	C	C
106	V	V	V	V	V	V
107	V	V	V	V	V	V
108	K	K	K	K	K	K
109	K	K	K	K	K	K
110	A	A	A	A	A	A
111	T	T	T	T	T	T
112	P	P	P	P	P	P
113	E	E	E	E	E	E
114	E	E	E	E	E	E
115	T	T	T	T	T	T
116	A	A	A	A	A	A
117	Y	Y	Y	Y	Y	Y
118	D	D	D	D	D	D
119	T	T	T	T	T	T
120	F	F	F	F	F	F
121	K	K	K	K	K	K
122	C	C	V	C	C	HY-, SUL/ALP
123	I	I	I	I	I	I
124	Y	Y	Y	Y	Y	Y
125	D	D	D	D	D	D
126	S	S	S	S	S	S
127	K	K	K	K	K	K
128	P	P	P	P	P	P
129	D	D	D	D	D	D
130	F	F	F	F	F	F
131	S	S	S	S	S	S
132	P	P	P	P	P	P
133	I	I	I	I	I	I
134	D	D	D	D	D	D
135						
136						
137						
138						

Tm 13.17	Consensus
T	ALP/ALH
E	ACD
K	K
I	I
I	ALP
N	ACD
K	K
C	C
A	ALP
V	V
K	K
R	BAS
D	ALP/ACD
T	T
V	ALP
E	E
E	E
T	T
V	ALP
F	ARO
N	ASX
T	T
F	F
K	K
C	HY- SUL/ALP
V	ALP
M	ARO/SUL
K	HY+ ACD/BAS
N	HY+, ALH/ACD
K	K
P	P
K	HY+ ACD/BAS
F	F
S	S
P	P
V	ALP
D	D

Tm P-B1	Tm P-B2	Consensus
S	S	ALP/ALH
E	E	ACD
K	K	K
I	I	I
V	V	ALP
E	E	ACD
K	K	K
C	C	C
T	T	ALP/ALH
V	V	V
T	T	HY+ ACD/ALH
E	E	HY+, BAS/ACD
D	D	ALP/ACD
T	T	T
P	P	ALP
E	E	E
D	D	ACD
T	T	T
A	A	ALP
F	F	ARO
E	E	ACD
V	V	ALH/ALP
T	T	ARO/ALH
K	K	K
C	C	HY-, SUL/ALP
V	V	ALP
L	L	ARO/SUL/ALP
K	K	HY+, ACD/BAS
D	D	HY+ ALH/ACD
K	K	K
P	P	P
N	N	HY+ ACD/BAS
F	F	F
F	F	ALH/ARO
G	G	ALP
D	D	ALP/ACD
L	L	ACD/ALP
F	F	F, OR GAP
V	V	V, OR GAP

Tm AFP-3	Consensus
V	ALP/ALH
D	ACD
D	HY+, BAS/ACD
L	ALP
V	ALP
A	ACD/ALP
K	K
C	C
A	ALP/ALH
V	V
K	HY+, ACD/ALH
K	HY+, BAS/ACD
D	ALP/ACD
T	T
P	ALP
Q	ACD
H	HY+, ACD/BAS
S	T
S	ALP/ALH
A	ARO/ALP
D	ACD
F	ALH/ALP/ARO
F	ARO/ALH/ARO
K	K
C	HY-, SUL/ALP
V	ALP
H	ARO/SUL/ALP/BAS
D	HY+, ACD/BAS
N	HY+, ALH/ACD
R	BAS
S	ALP/ALH
	HY+, ACD/BAS OR GAP
	F OR GAP
	ALH/ARO OR GAP
	ALP OR GAP
	ALP/ACD OR GAP
	ACD/ALP OR GAP
	F OR GAP
	V, OR GAP

GENERAL	SUBSTITUTIONS - most to least common			
V	S	T		
D	E			
K	D			
I	L			
V	I			
Q	E	N	A	
K				
C				
V	A	T		
V				
K	T			
K	E	R		
D	A			
T				
P	V			
E	Q			
E	D	H		
T	S			
A	V	S		
Y	F	A		
D	E	N		
T	V	F		
F	T			
K				
C	V	(3 4 is the only variant in the family)		
V	I			
Y	L	M	H	
D	K			
S	N	D		
K	R			
P	S			
D	N	K		
F				
S	F			
P	G			
I	D	V		
D	L			
F				
V				

FIG. 7.3 Cont.

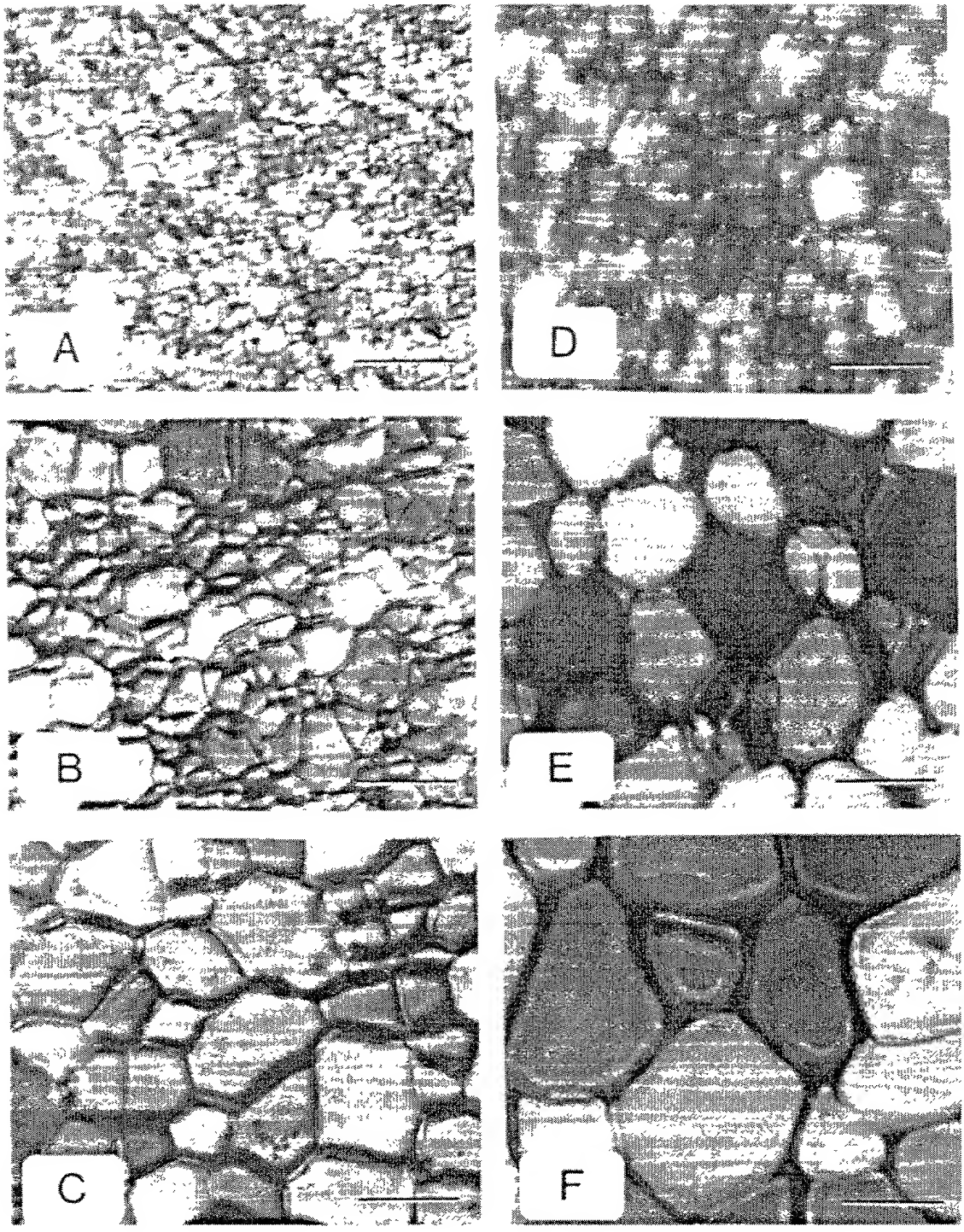

FIG. 8.0

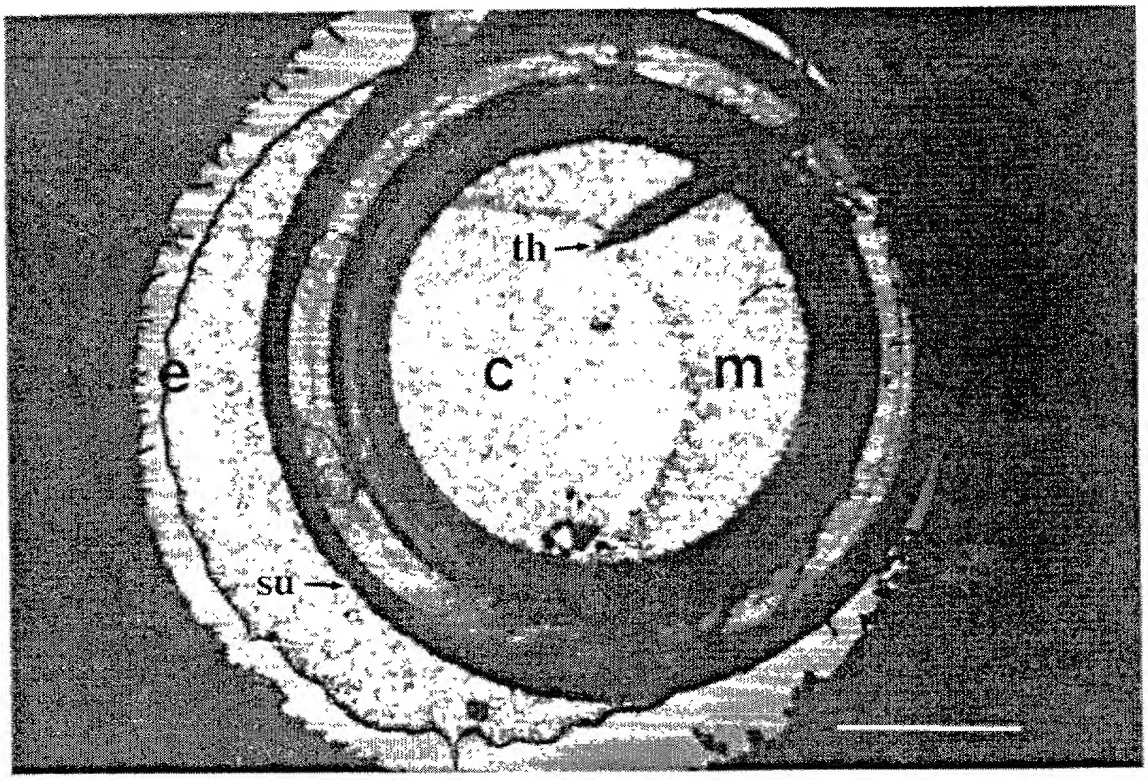

FIG. 8.1a

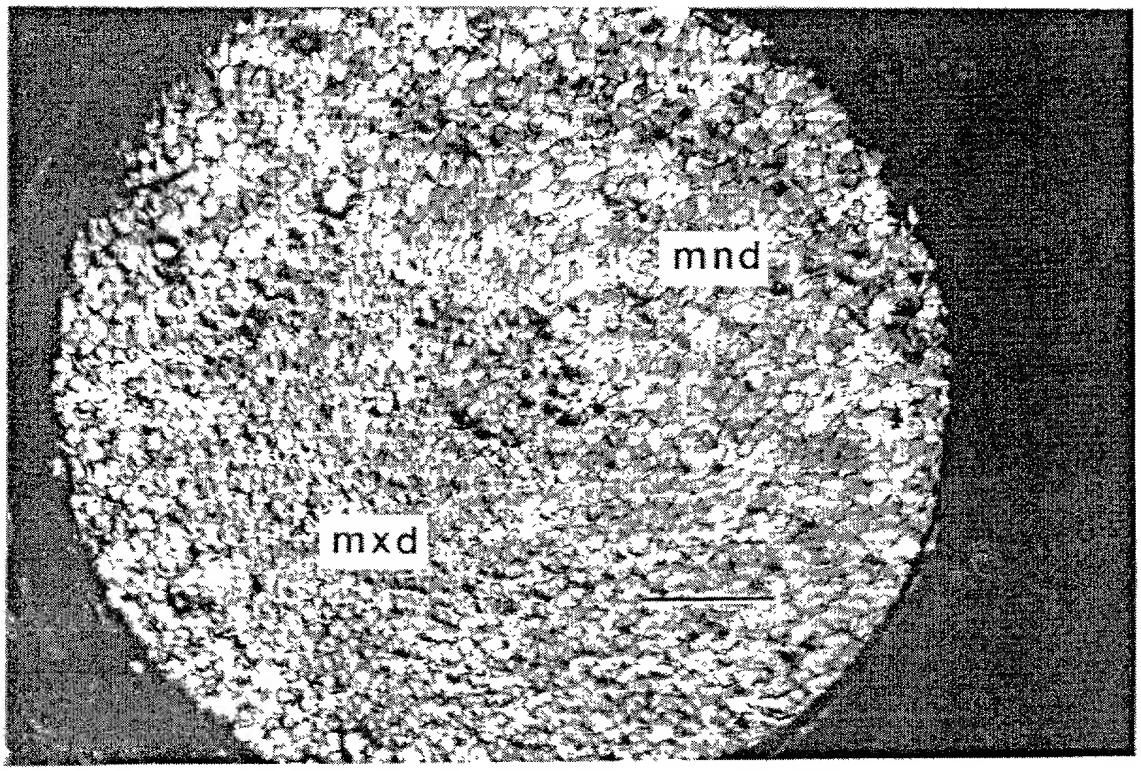

FIG. 8.1b

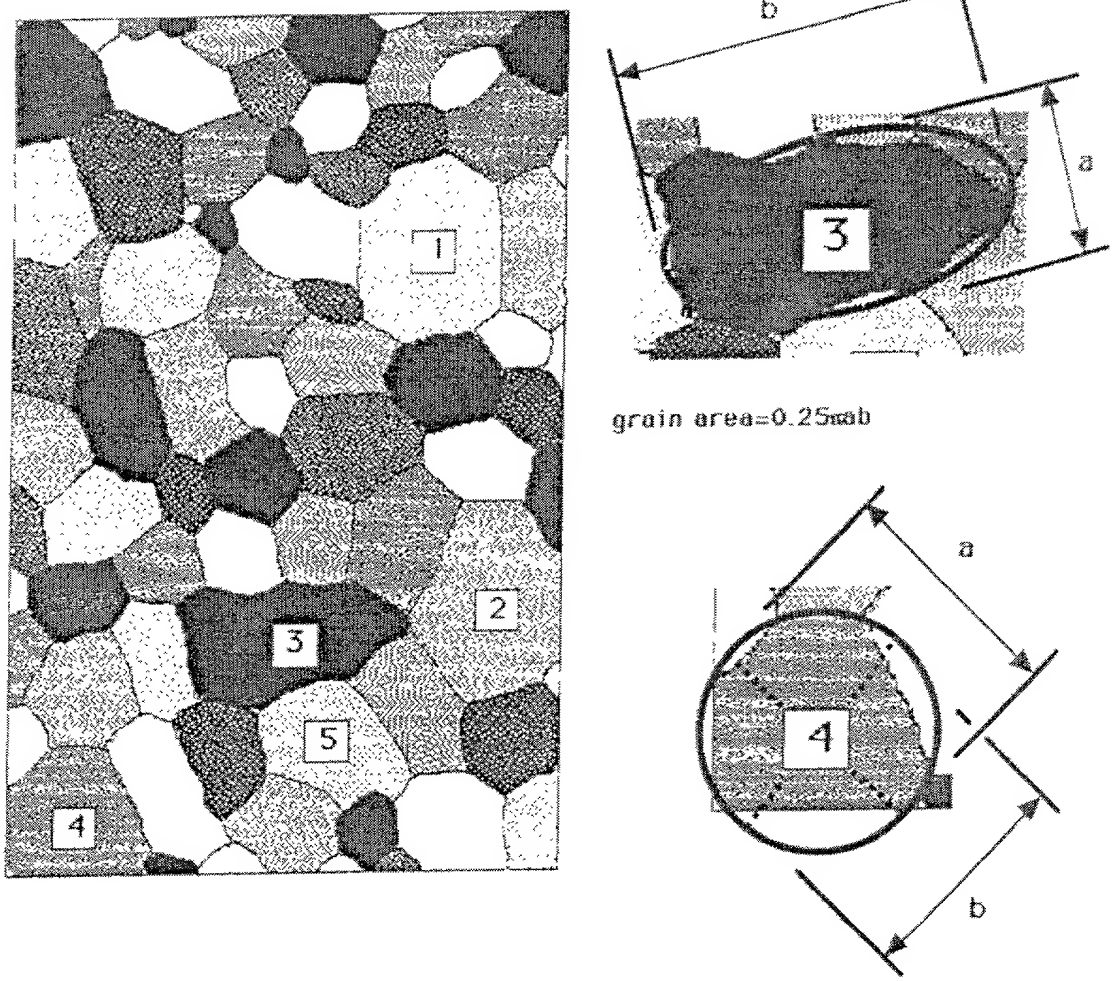

FIG. 8.2

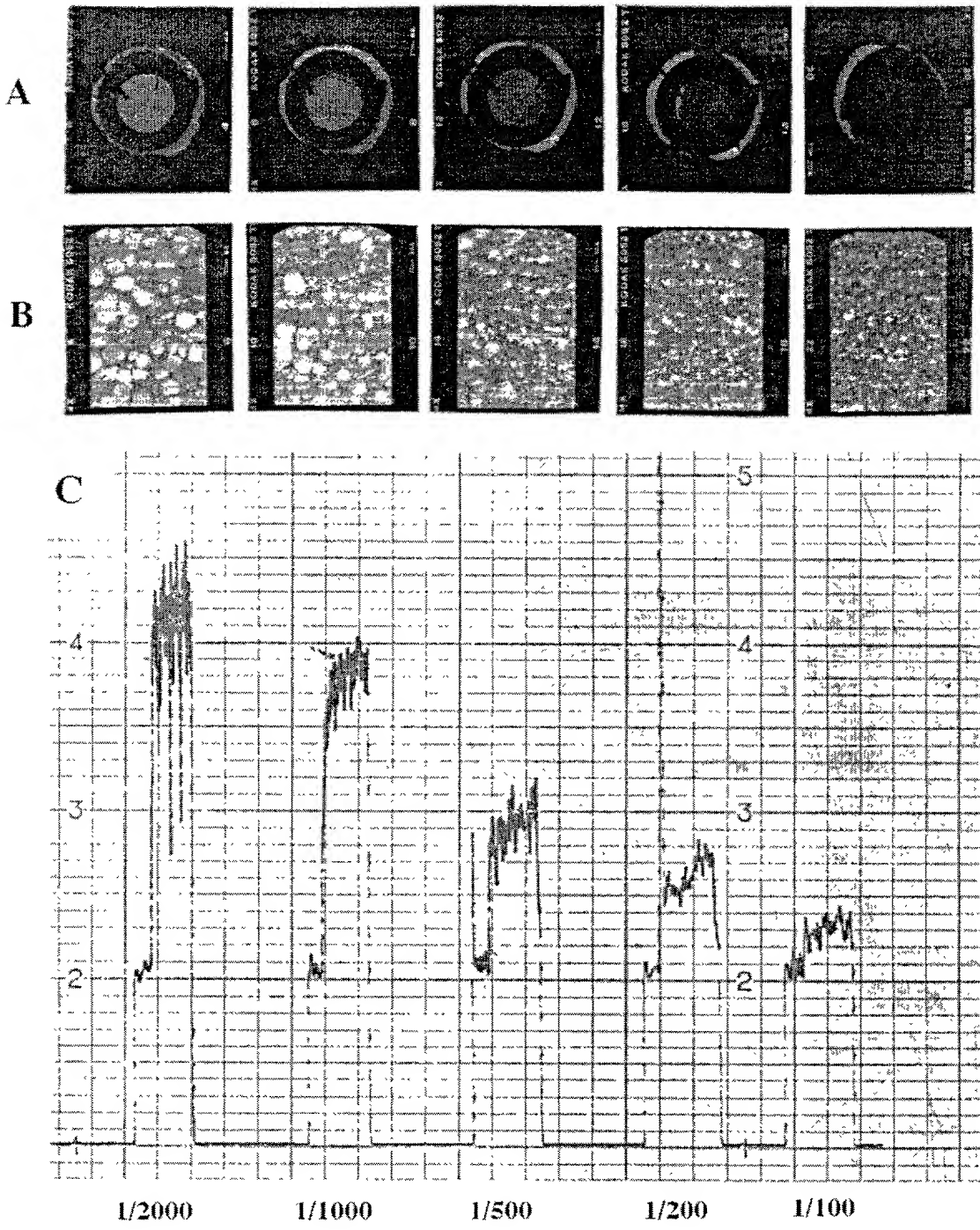

FIG. 8.3

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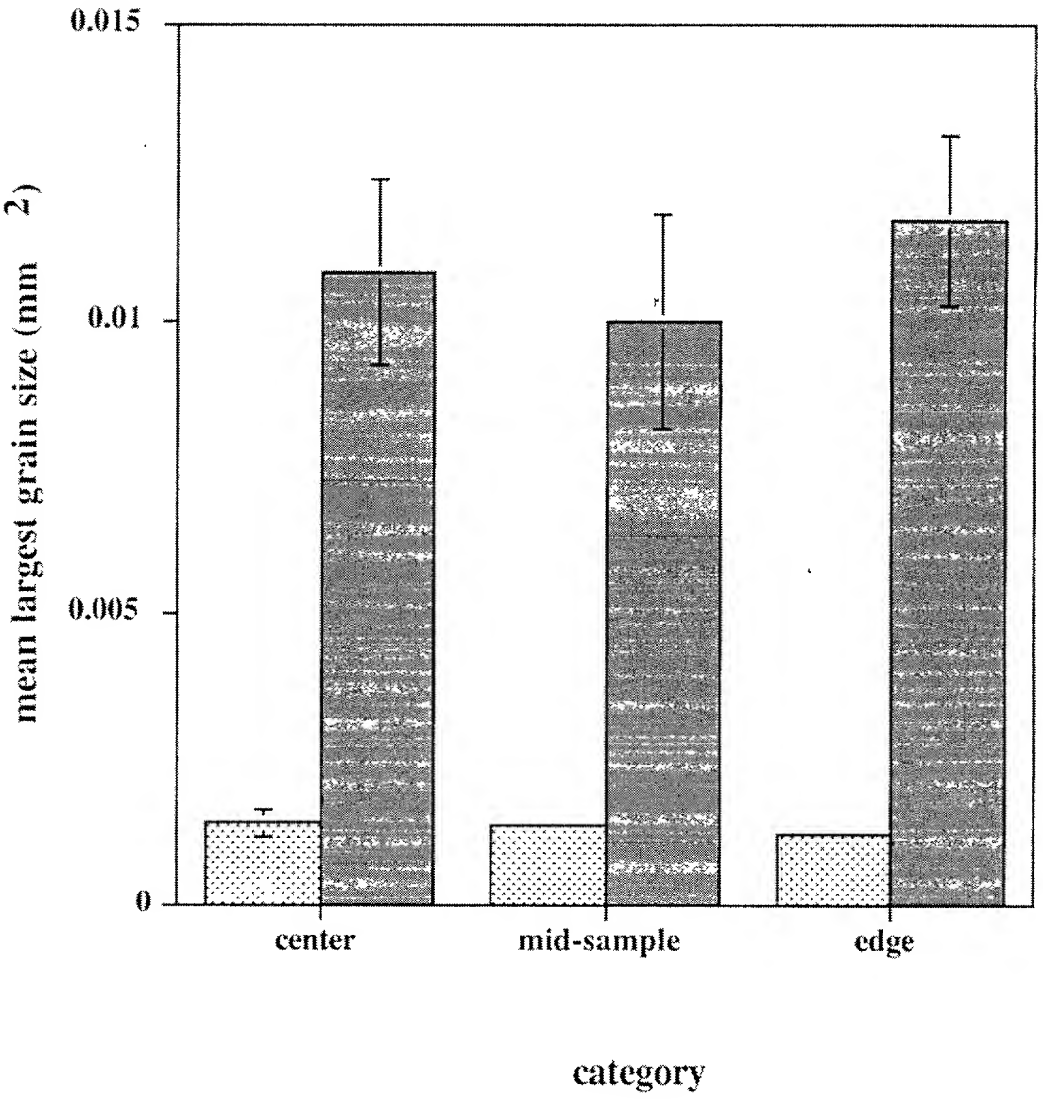

FIG. 8.4a

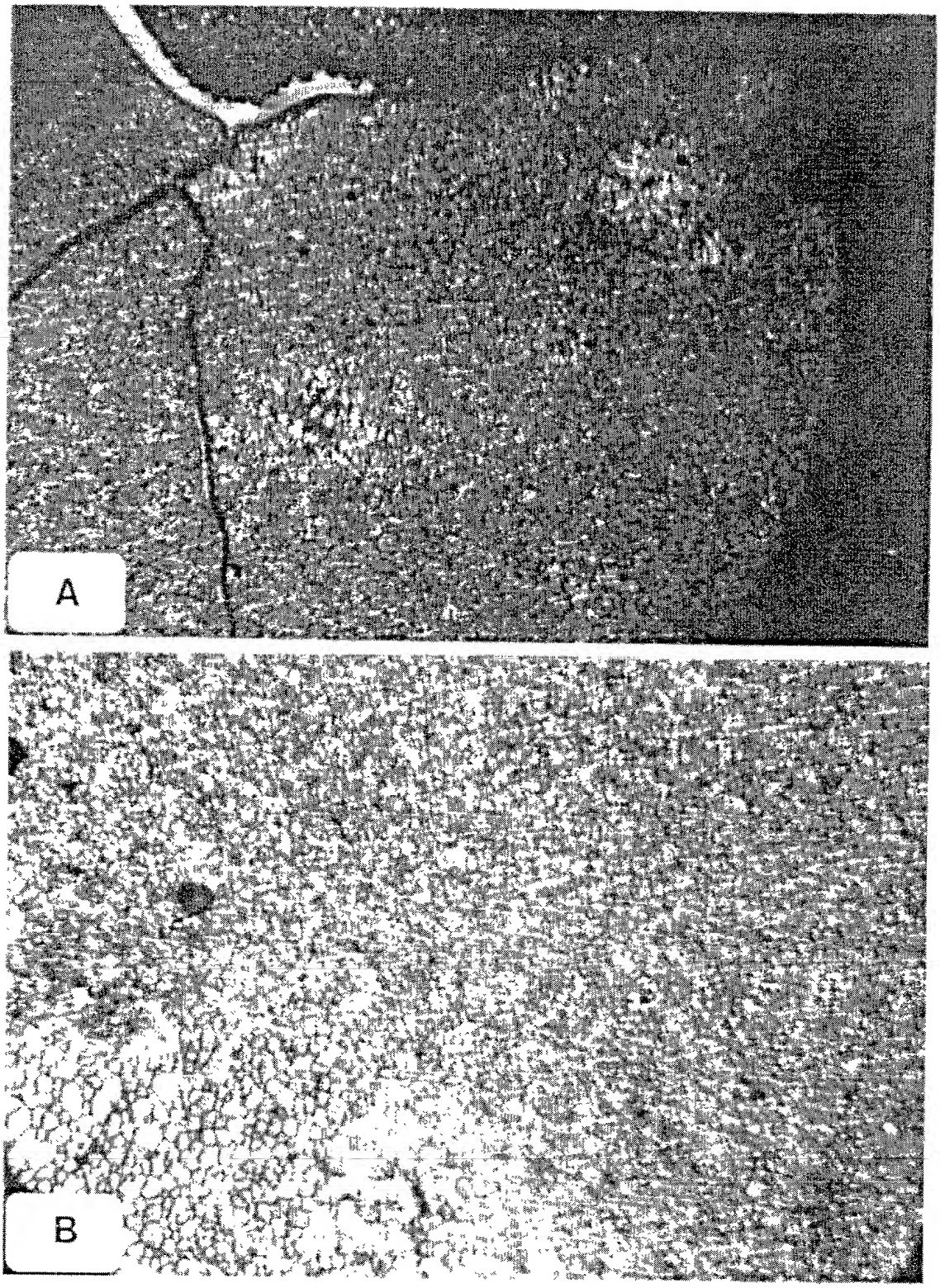

FIG. 8.4b

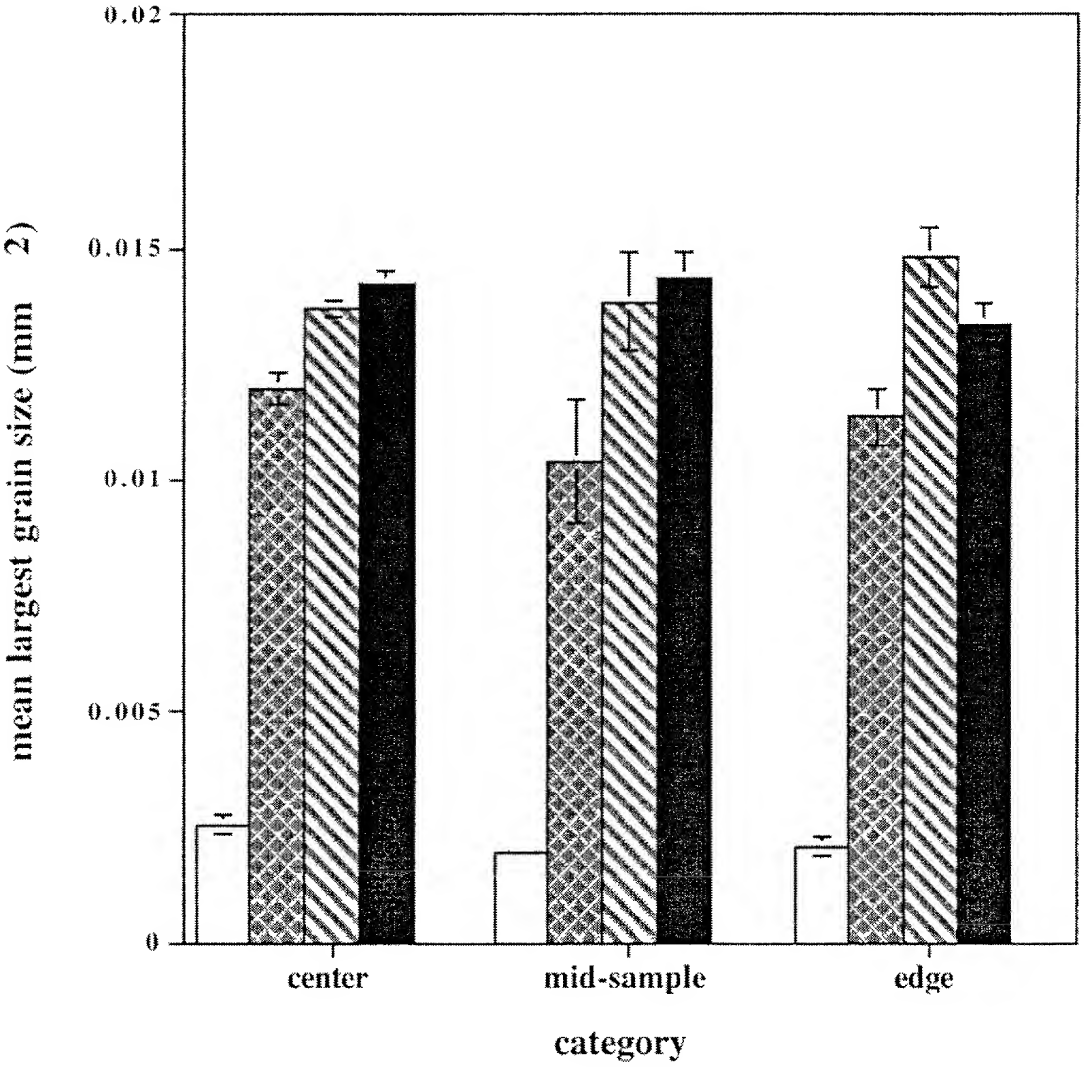
0.02
0.015
0.01
0.005
0
mean largest grain size (mm 2)
center
mid-sample
edge
category

FIG. 8.5a

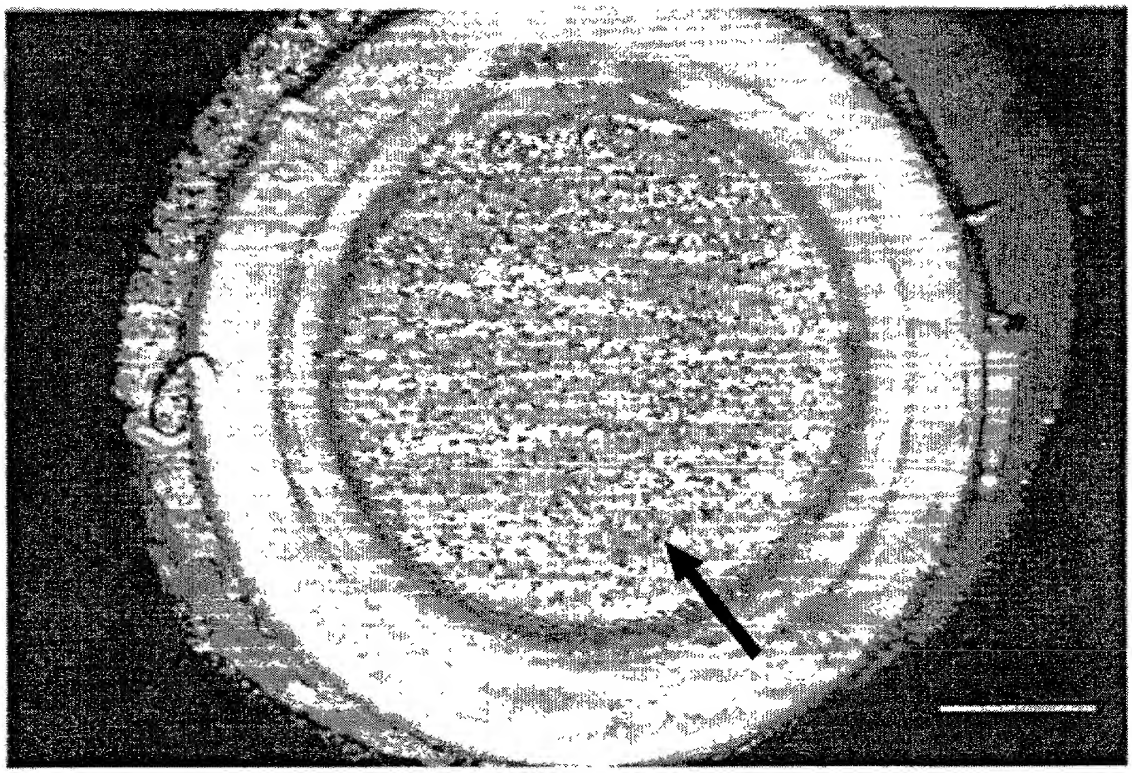

FIG. 8.5b

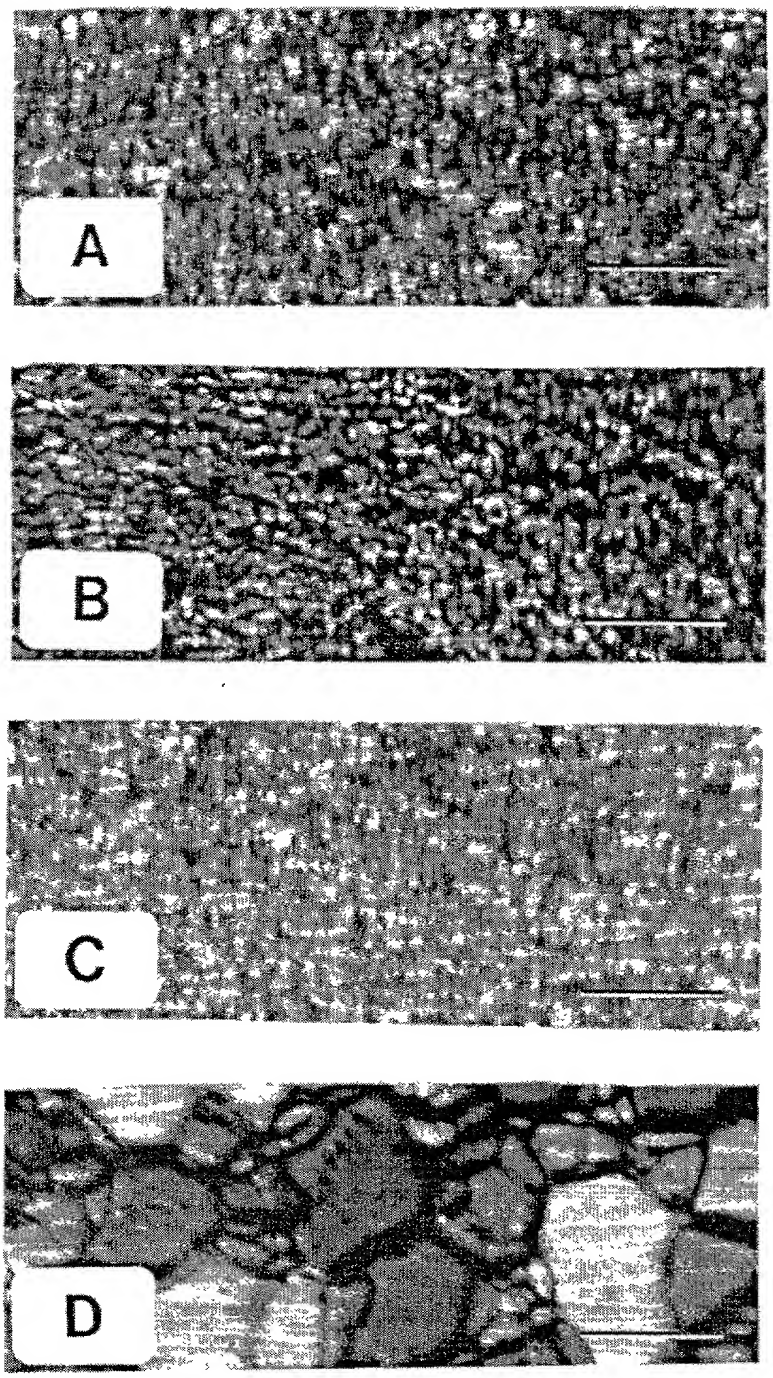

FIG. 8.6

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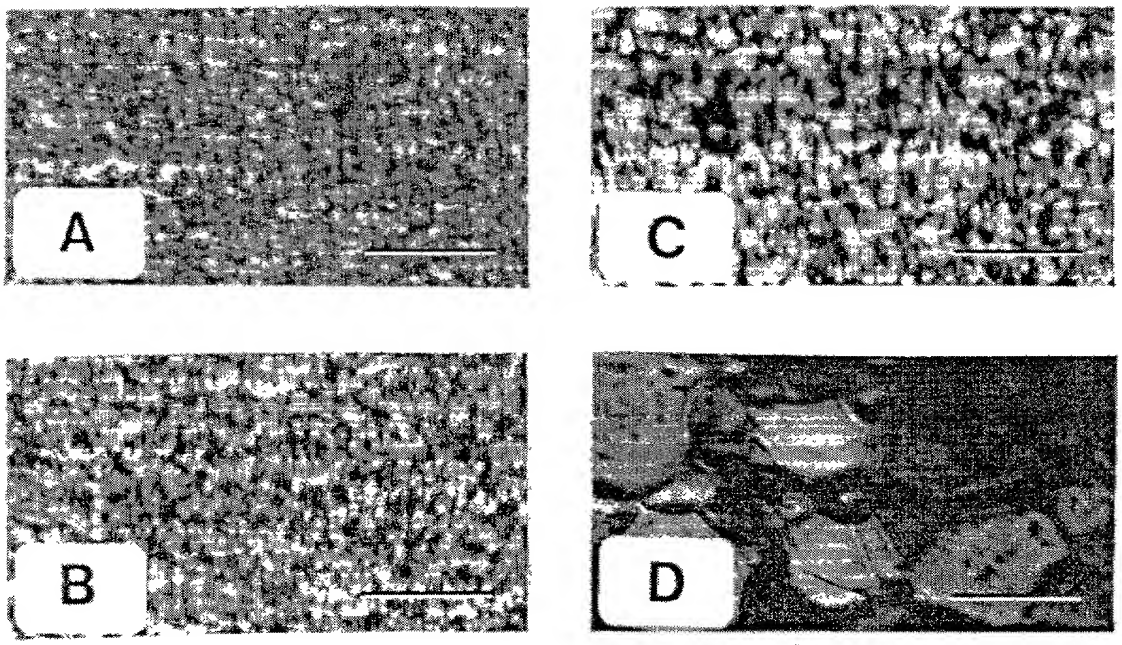

FIG. 8.7

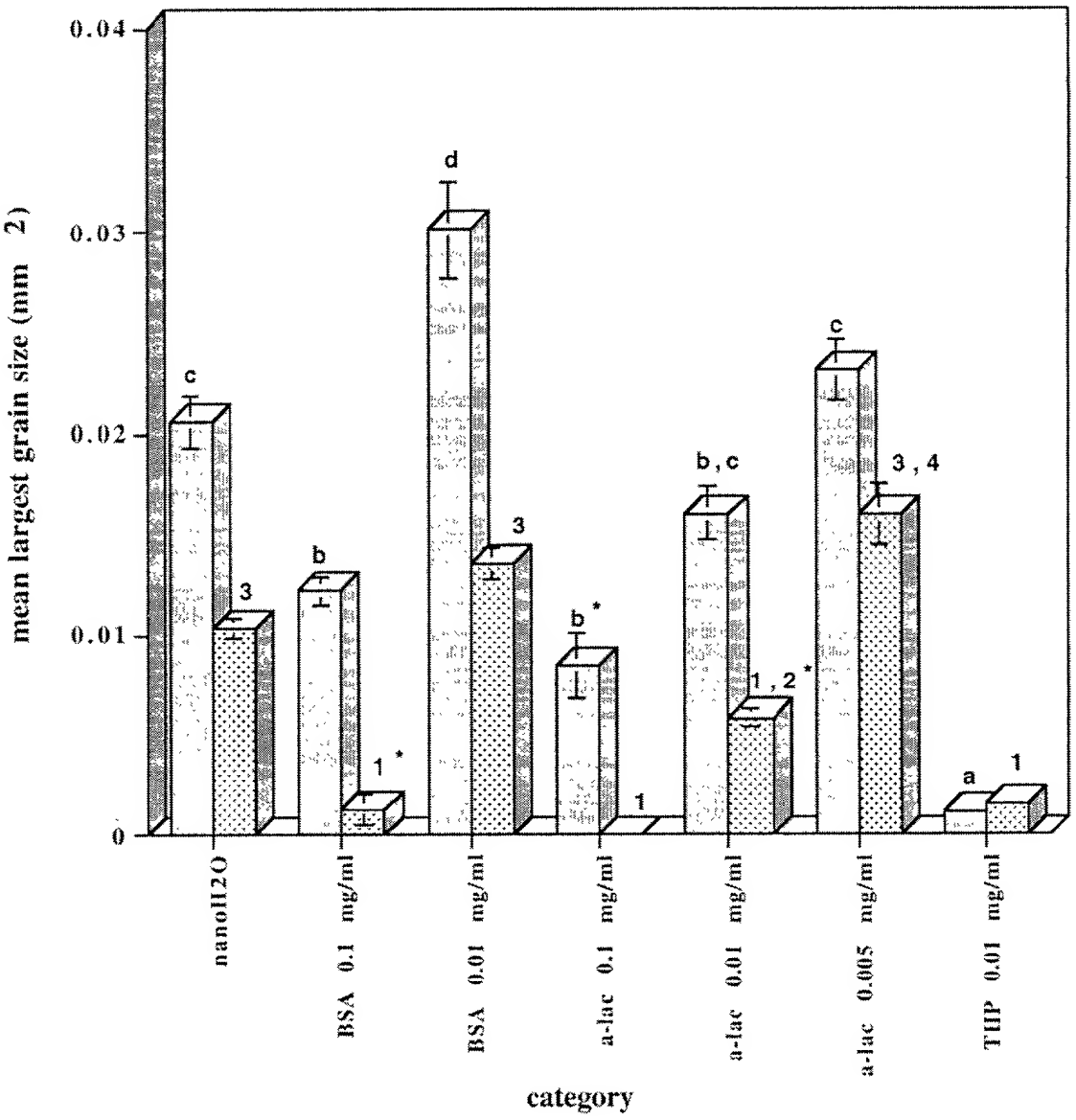
mean largest grain size (mm 2)
0.04
0.03
0.02
0.01
0
c
3
b
1 *
d
3
b *
1
b , c
1 , 2 *
c
3 , 4
a
1
nanoH2O
BSA 0.1 mg/ml
BSA 0.01 mg/ml
a-lac 0.1 mg/ml
a-lac 0.01 mg/ml
a-lac 0.005 mg/ml
THP 0.01 mg/ml
category

FIG. 8.8

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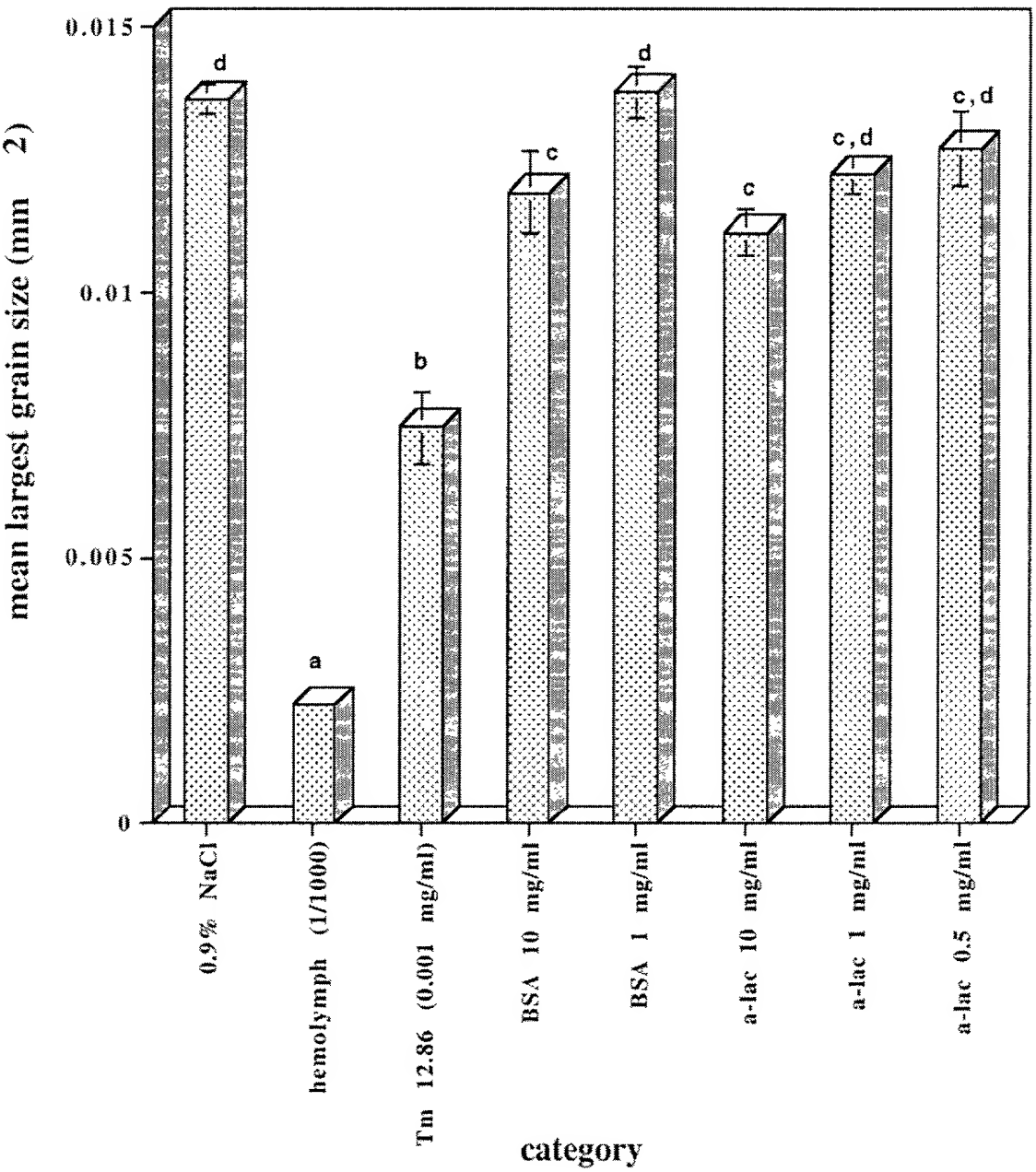

FIG. 8.9

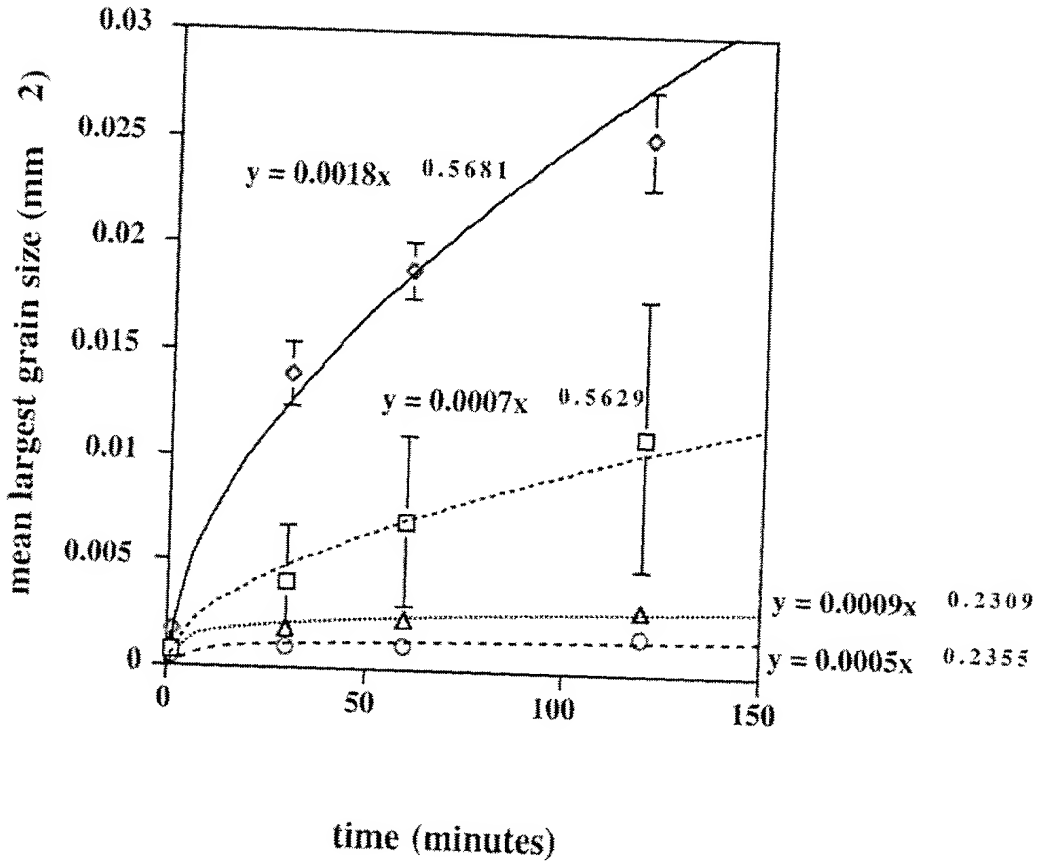
0.03
0.025
0.02
0.015
0.01
0.005
0
mean largest grain size (mm 2)
y = 0.0018x 0.5681
y = 0.0007x 0.5629
y = 0.0009x 0.2309
y = 0.0005x 0.2355
0
50
100
150
time (minutes)

FIG. 8.10

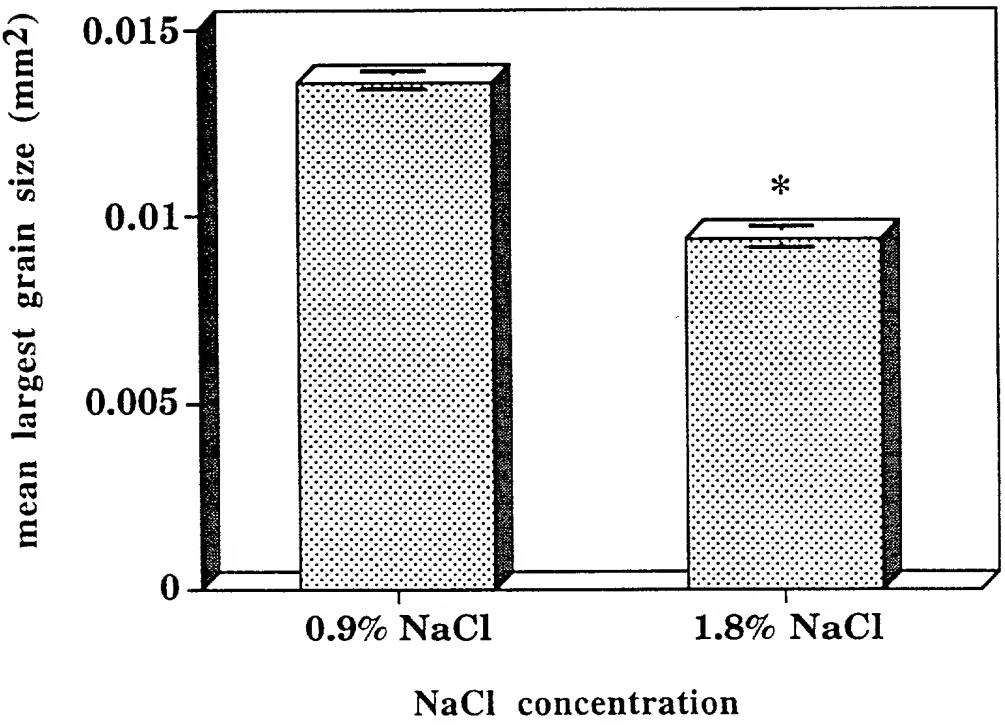
mean largest grain size (mm2)
0.015
0.01
0.005
0
*
0.9% NaCl
1.8% NaCl
NaCl concentration

FIG. 8.11

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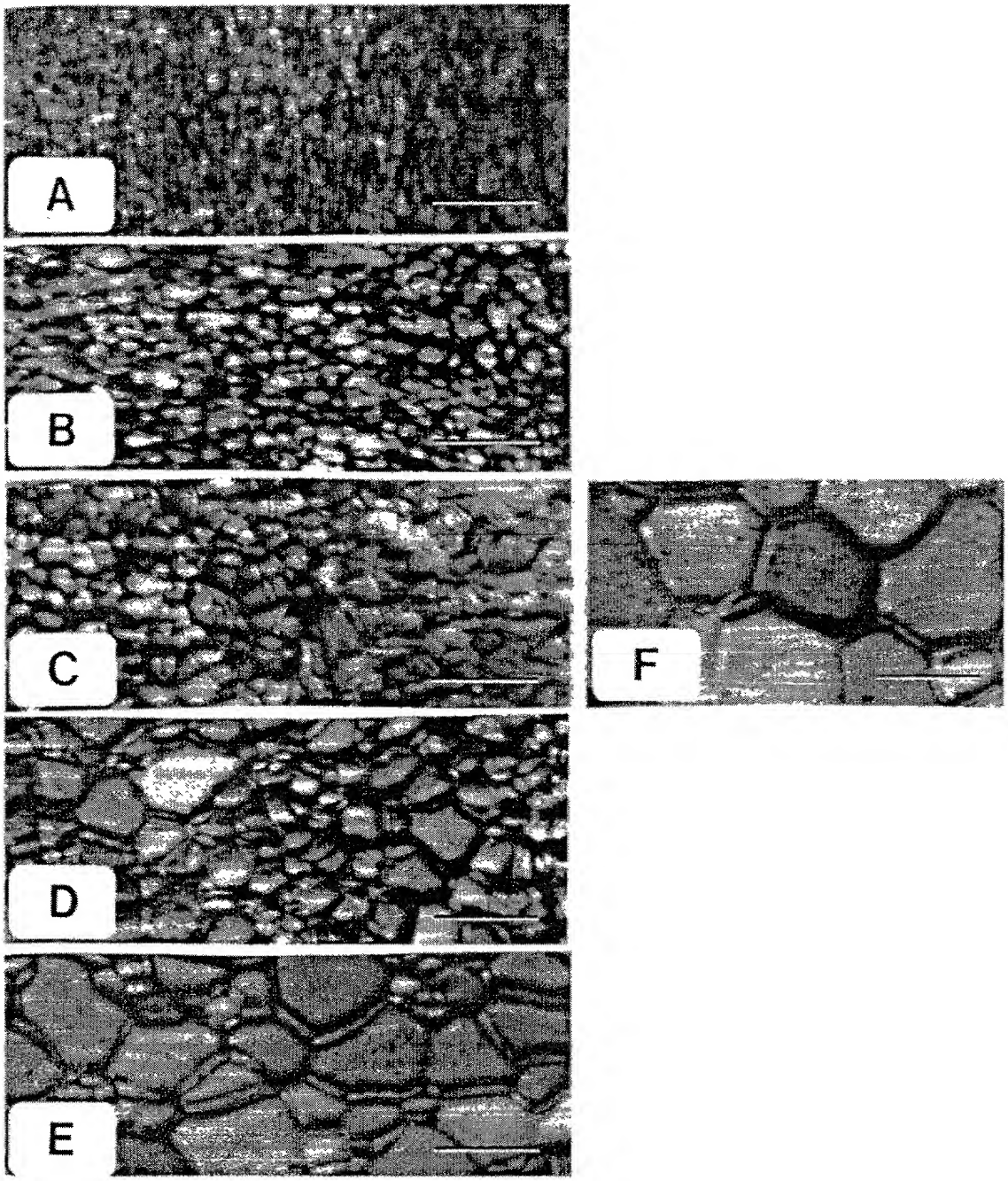


FIG. 8.12

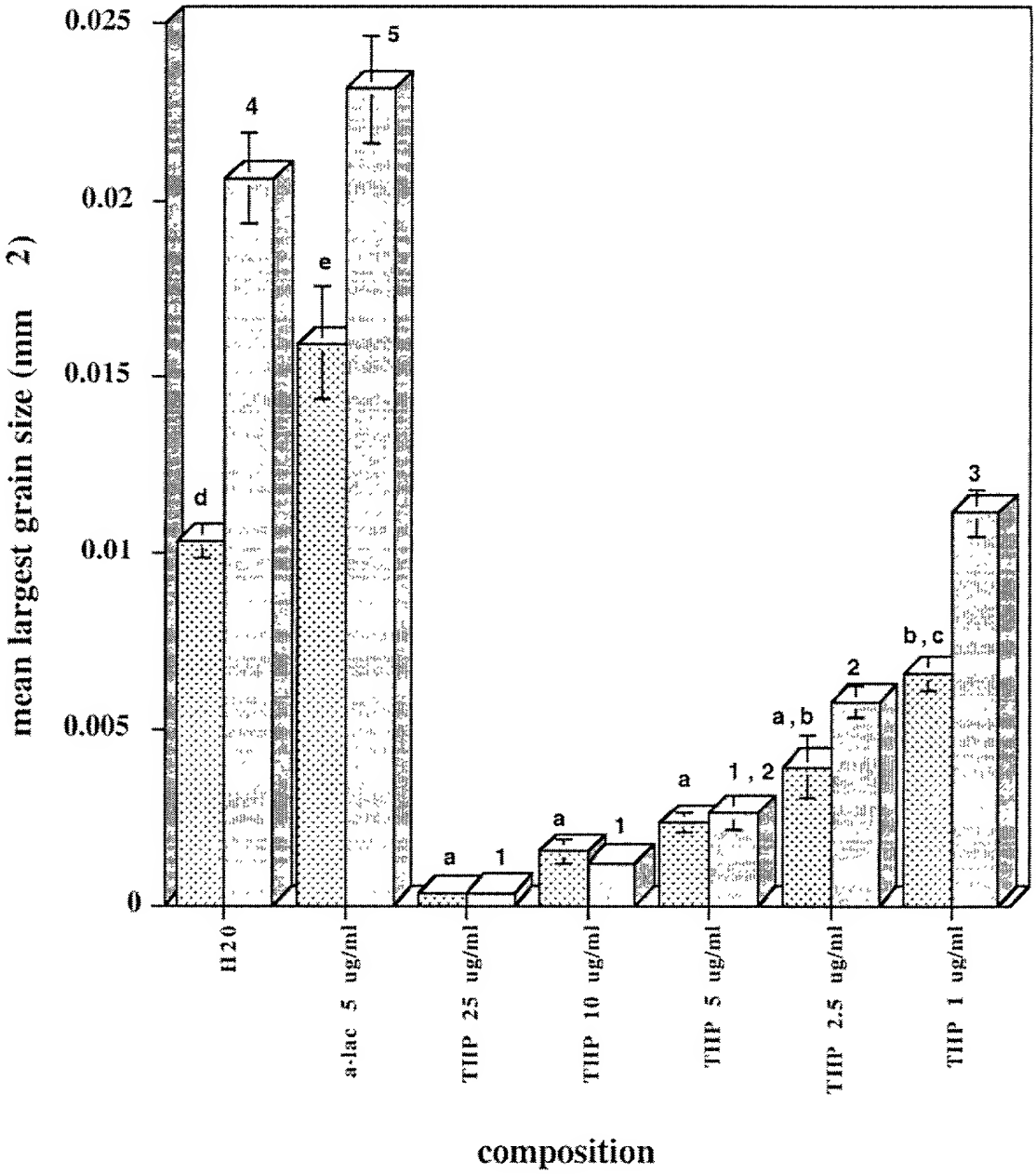

0.025
0.02
0.015
0.01
0.005
0
mean largest grain size (mm 2)
d
4
e
5
a
1
a
1
a
1, 2
a, b
2
b, c
3
H20
a-lac 5 ug/ml
THP 25 ug/ml
THP 10 ug/ml
THP 5 ug/ml
THP 2.5 ug/ml
THP 1 ug/ml
composition

FIG. 8.13

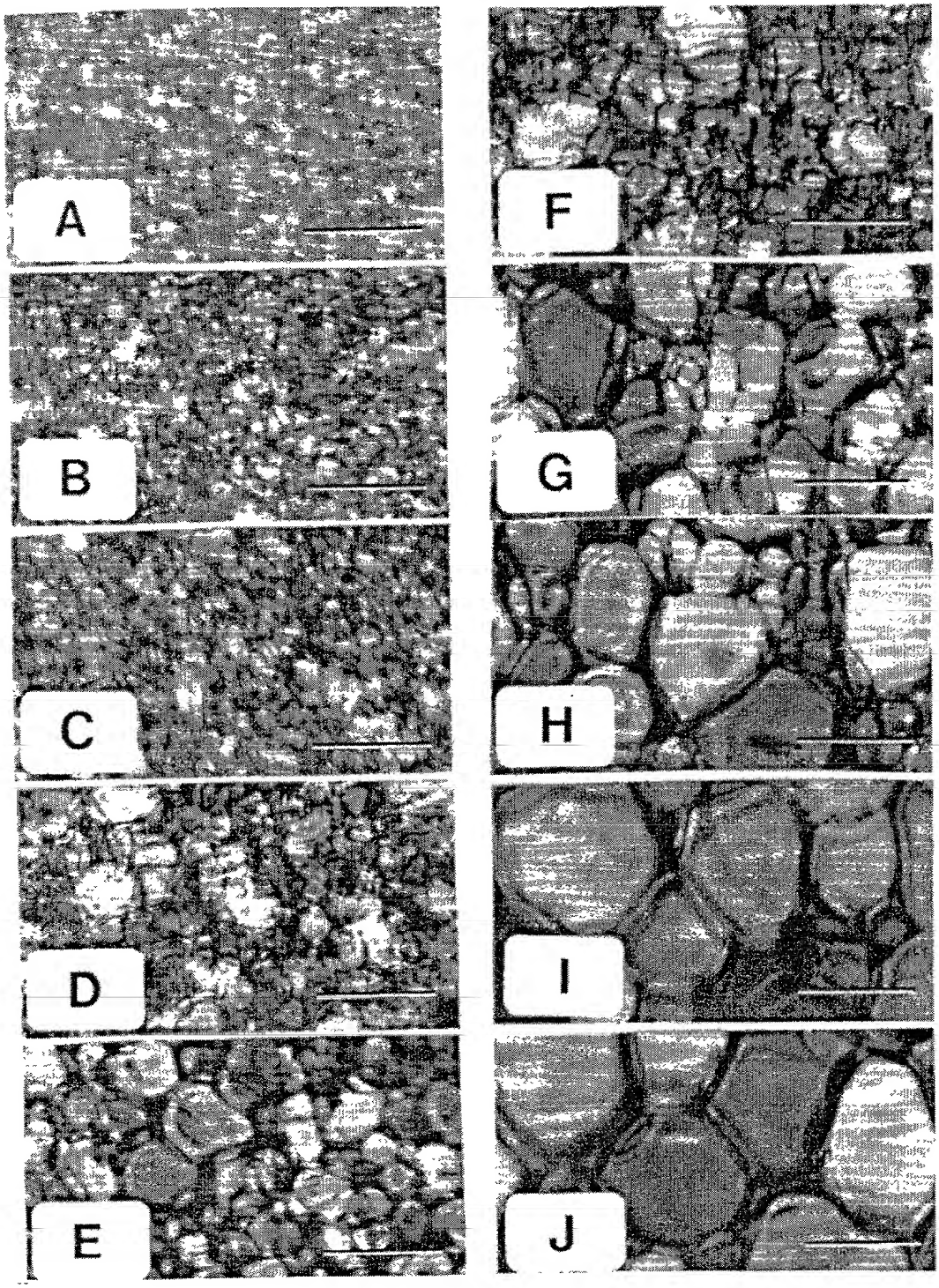


FIG. 8.14

TO2090 96292860

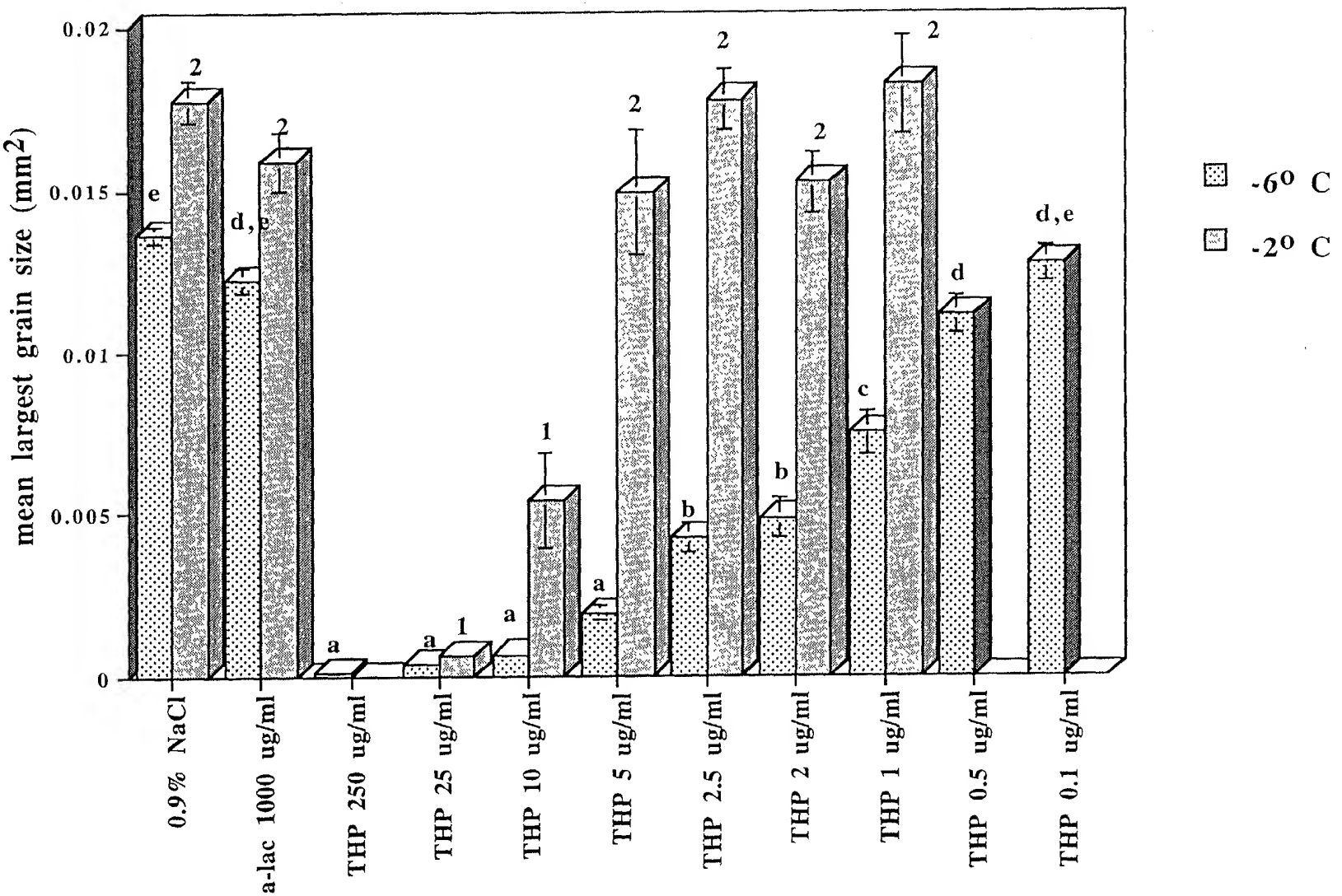


FIG. 8.15

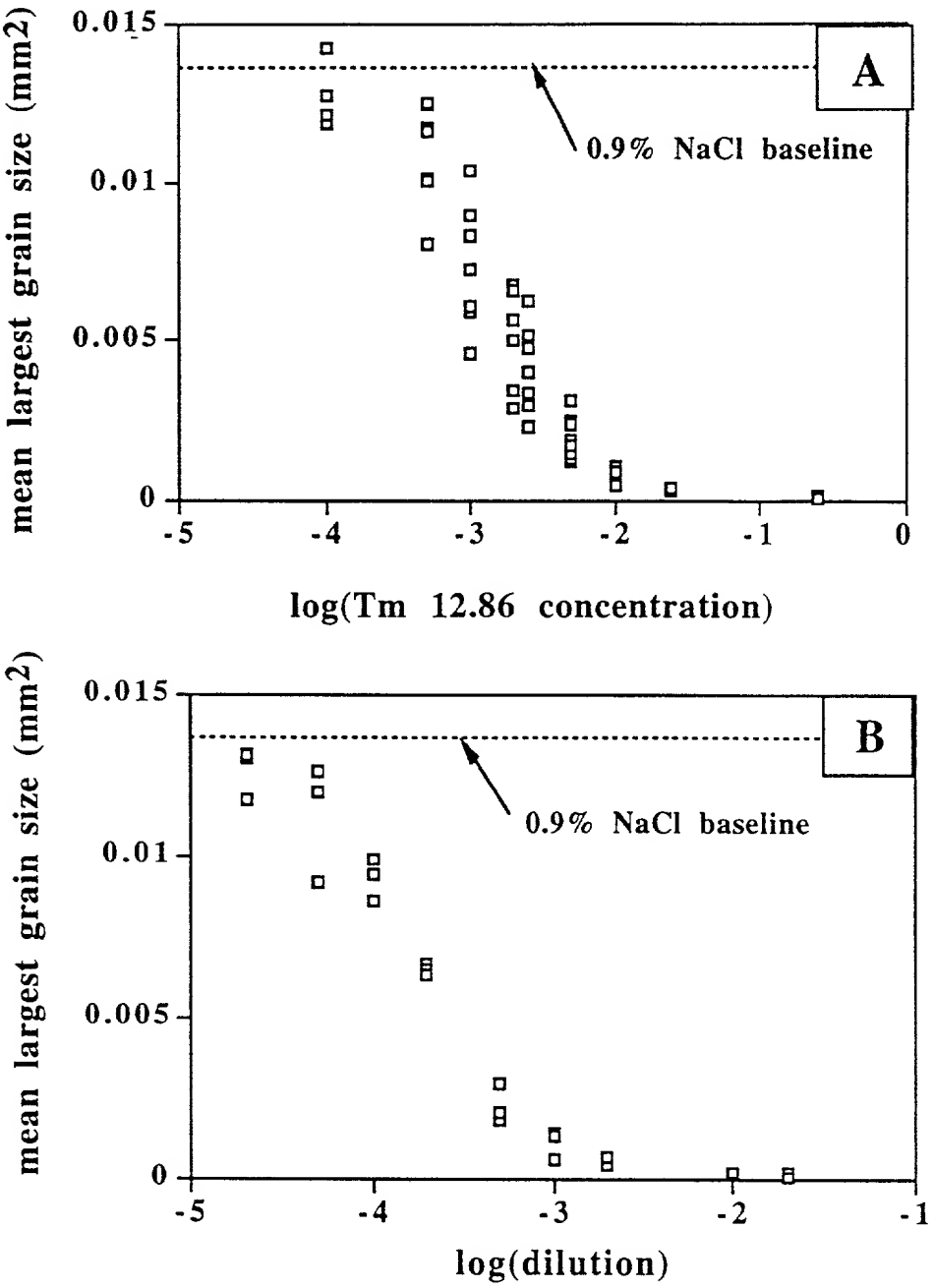

A
0.9% NaCl baseline
mean largest grain size (mm2)
0.015
0.01
0.005
0
-5
-4
-3
-2
-1
0
log(Tm 12.86 concentration)
B
0.9% NaCl baseline
mean largest grain size (mm2)
0.015
0.01
0.005
0
-5
-4
-3
-2
-1
log(dilution)

FIG. 8.16

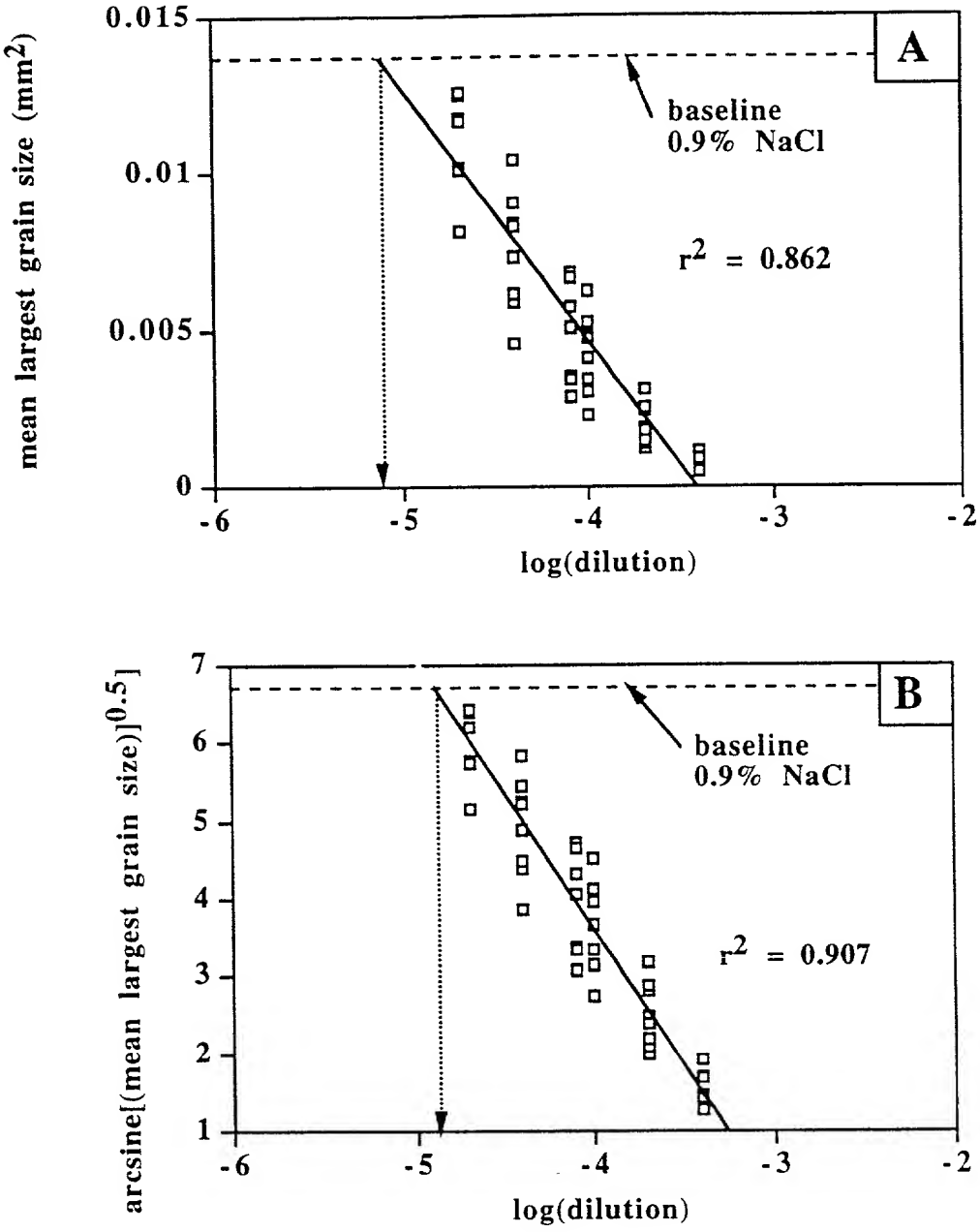

A
baseline
0.9% NaCl
r^2 = 0.862
mean largest grain size (mm^2)
0.015
0.01
0.005
0
-6
-5
-4
-3
-2
log(dilution)
B
baseline
0.9% NaCl
r^2 = 0.907
arcsine[(mean largest grain size)]$^{0.5}$]
7
6
5
4
3
2
1
-6
-5
-4
-3
-2
log(dilution)

FIG. 8.17

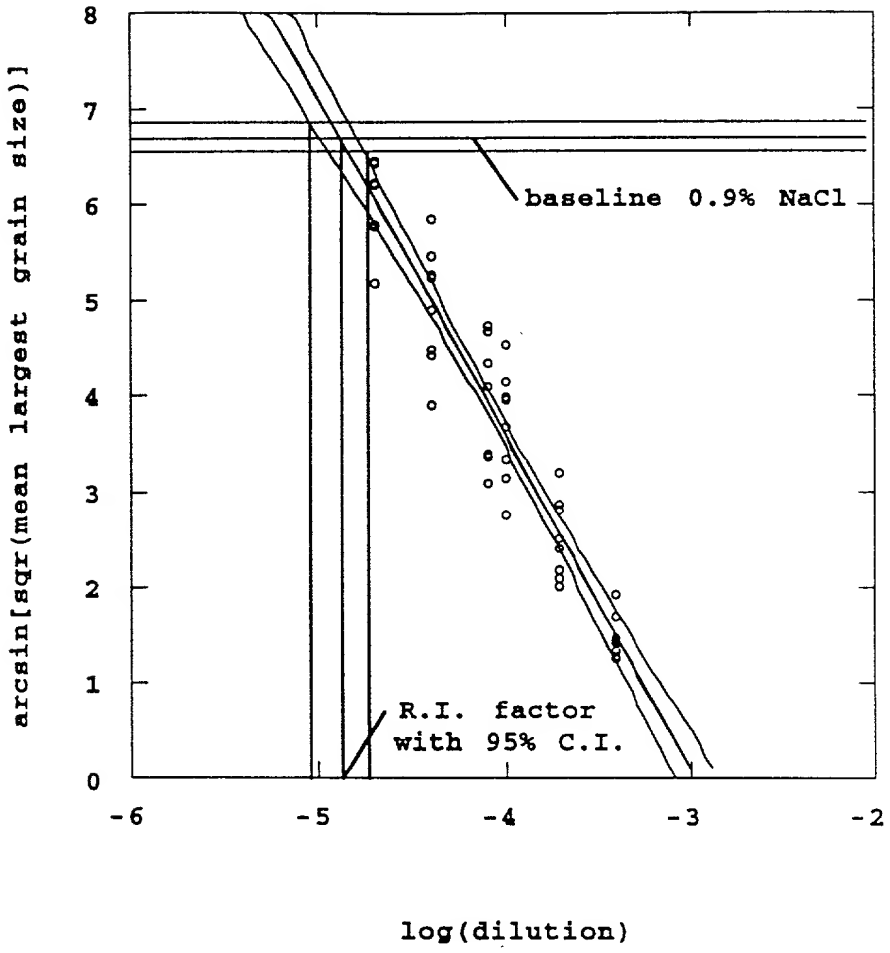

arcsin[sqr(mean largest grain size)]
8
7
6
5
4
3
2
1
0
-6
-5
-4
-3
-2
baseline 0.9% NaCl
R.I. factor
with 95% C.I.
log(dilution)

FIG. 8.18

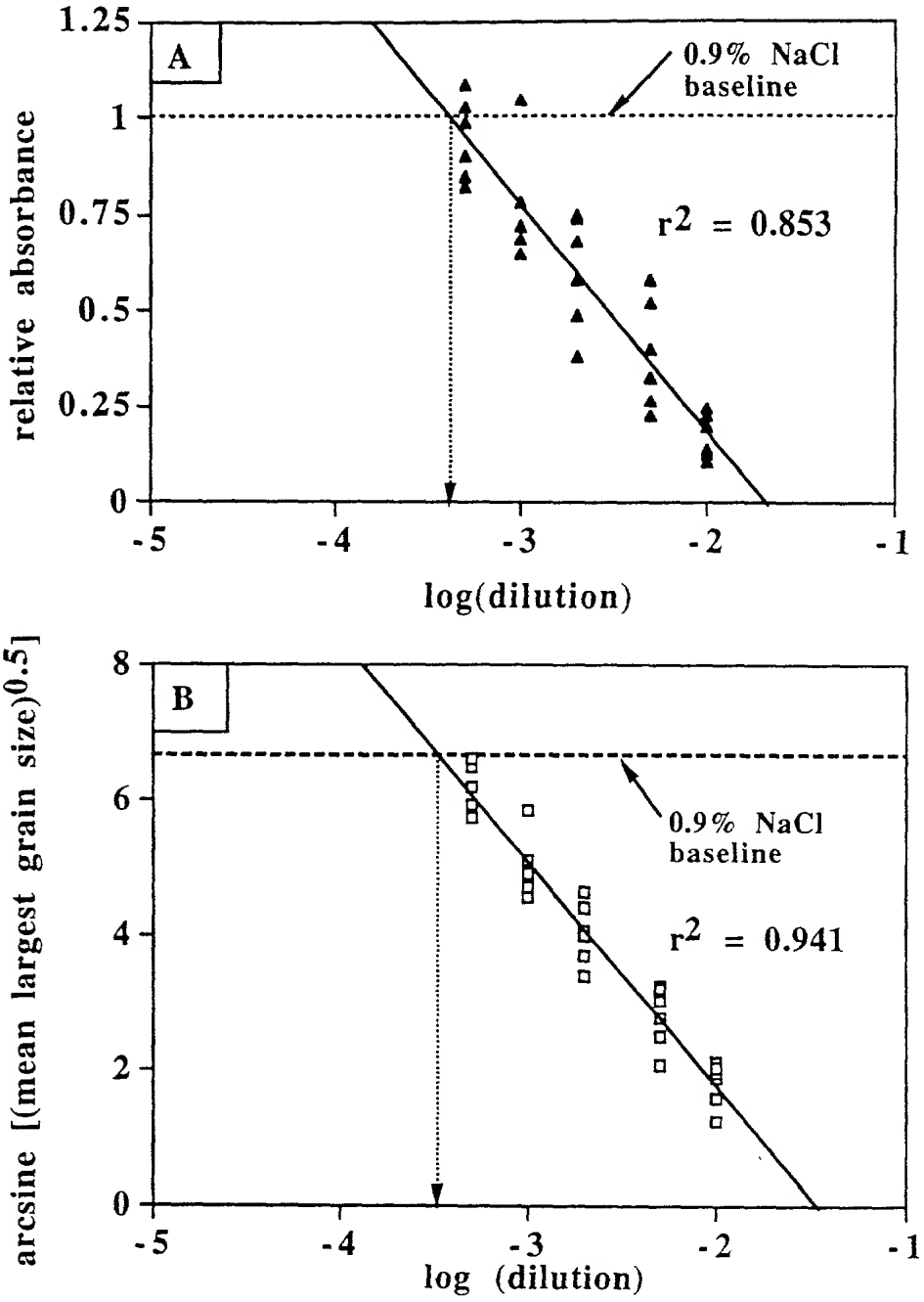

A
0.9% NaCl baseline
r^2 = 0.853
relative absorbance
1.25
1
0.75
0.5
0.25
0
-5
-4
-3
-2
-1
log(dilution)
B
0.9% NaCl baseline
r^2 = 0.941
arcsine [(mean largest grain size)0.5]
8
6
4
2
0
-5
-4
-3
-2
-1
log (dilution)

FIG. 8.19

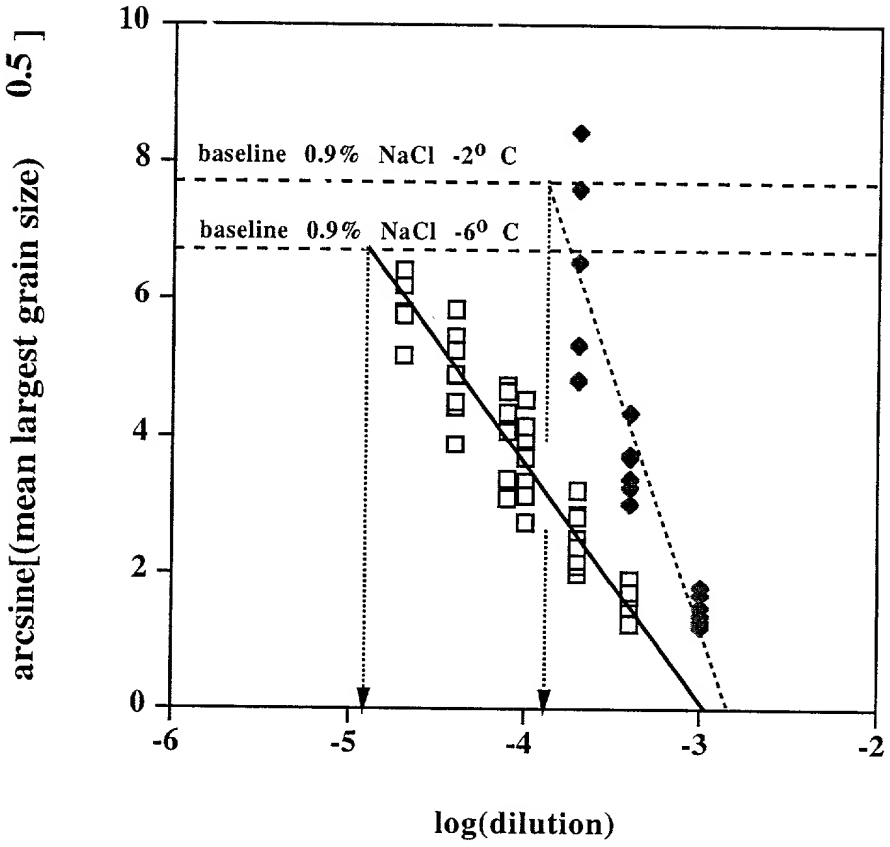

arcsine[(mean largest grain size) 0.5]
10
8
6
4
2
0
baseline 0.9% NaCl -2° C
baseline 0.9% NaCl -6° C
-6
-5
-4
-3
-2
log(dilution)

FIG. 8.20

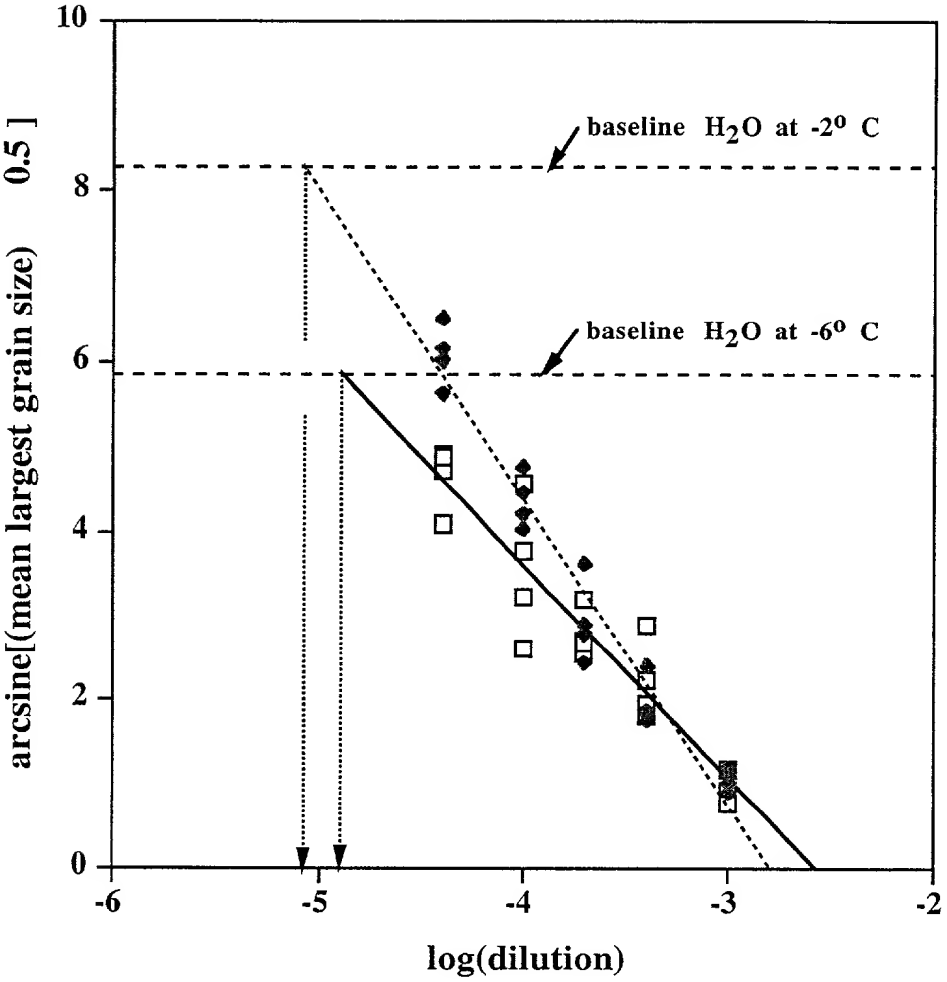

10
8
6
4
2
0
-6
-5
-4
-3
-2
arcsine[(mean largest grain size) 0.5]
log(dilution)
baseline H2O at -2° C
baseline H2O at -6° C

FIG. 8.21

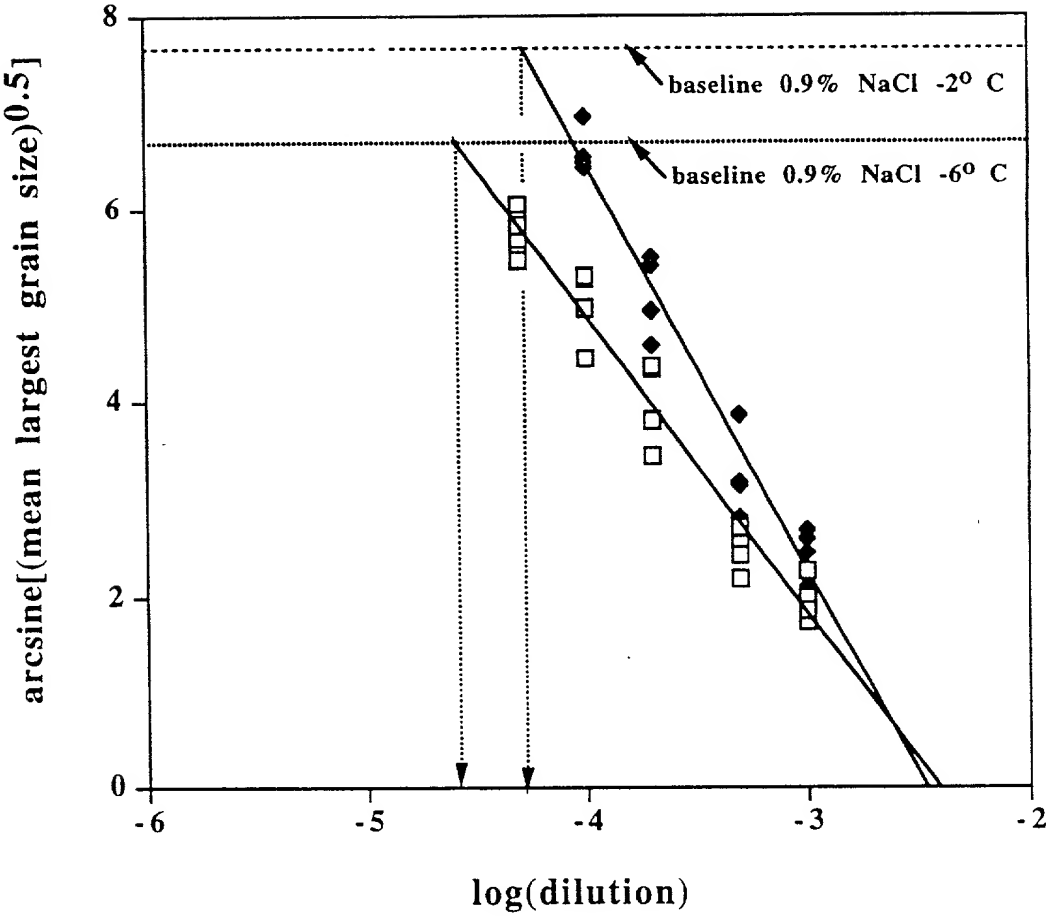

arcsine[(mean largest grain size)0.5]
8
6
4
2
0
-6
-5
-4
-3
-2
log(dilution)
baseline 0.9% NaCl -2° C
baseline 0.9% NaCl -6° C

FIG. 8.22

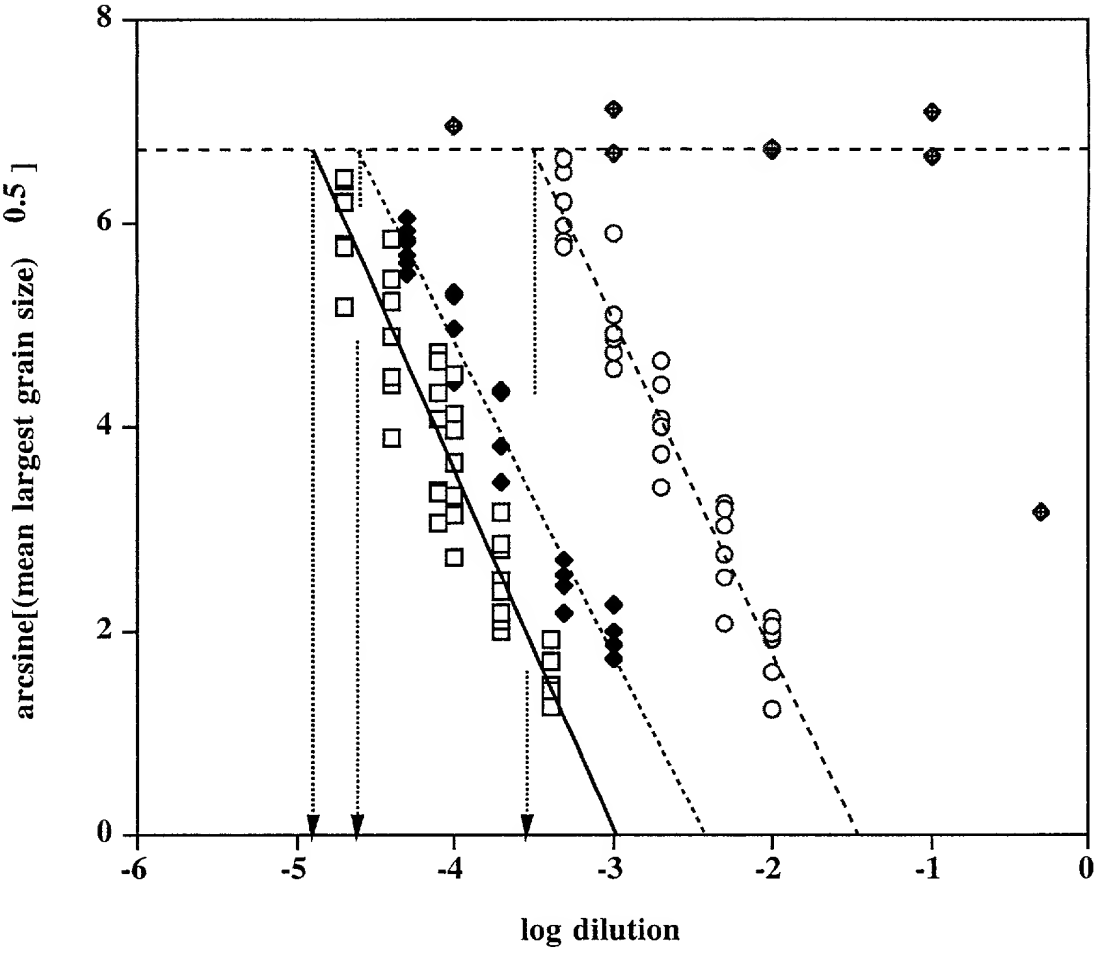

8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
arcsine[(mean largest grain size) 0.5]
log dilution

FIG. 8.23

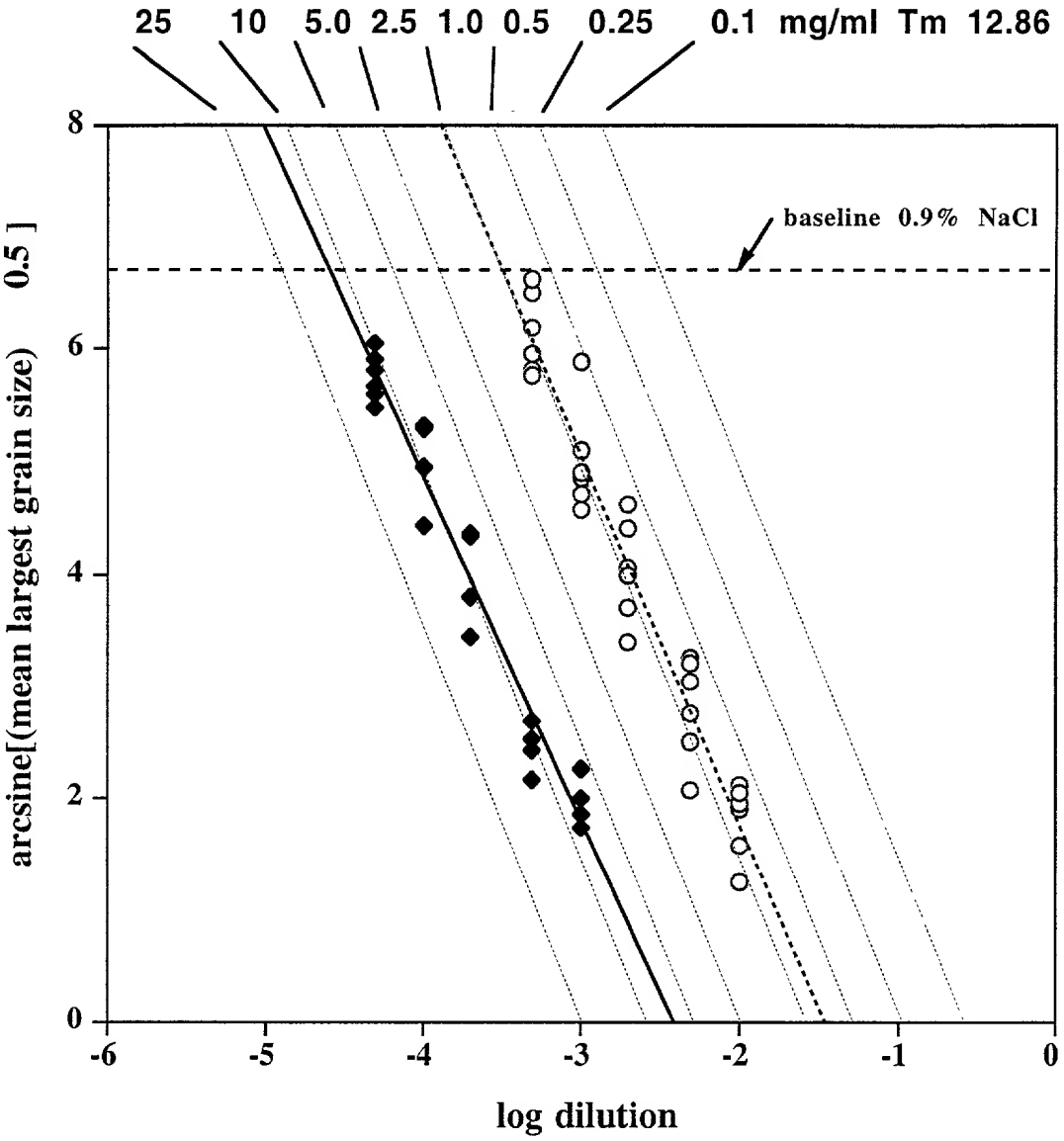

25 10 5.0 2.5 1.0 0.5 0.25 0.1 mg/ml Tm 12.86
baseline 0.9% NaCl
arcsine[(mean largest grain size) 0.5]
log dilution
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0

FIG. 8.24

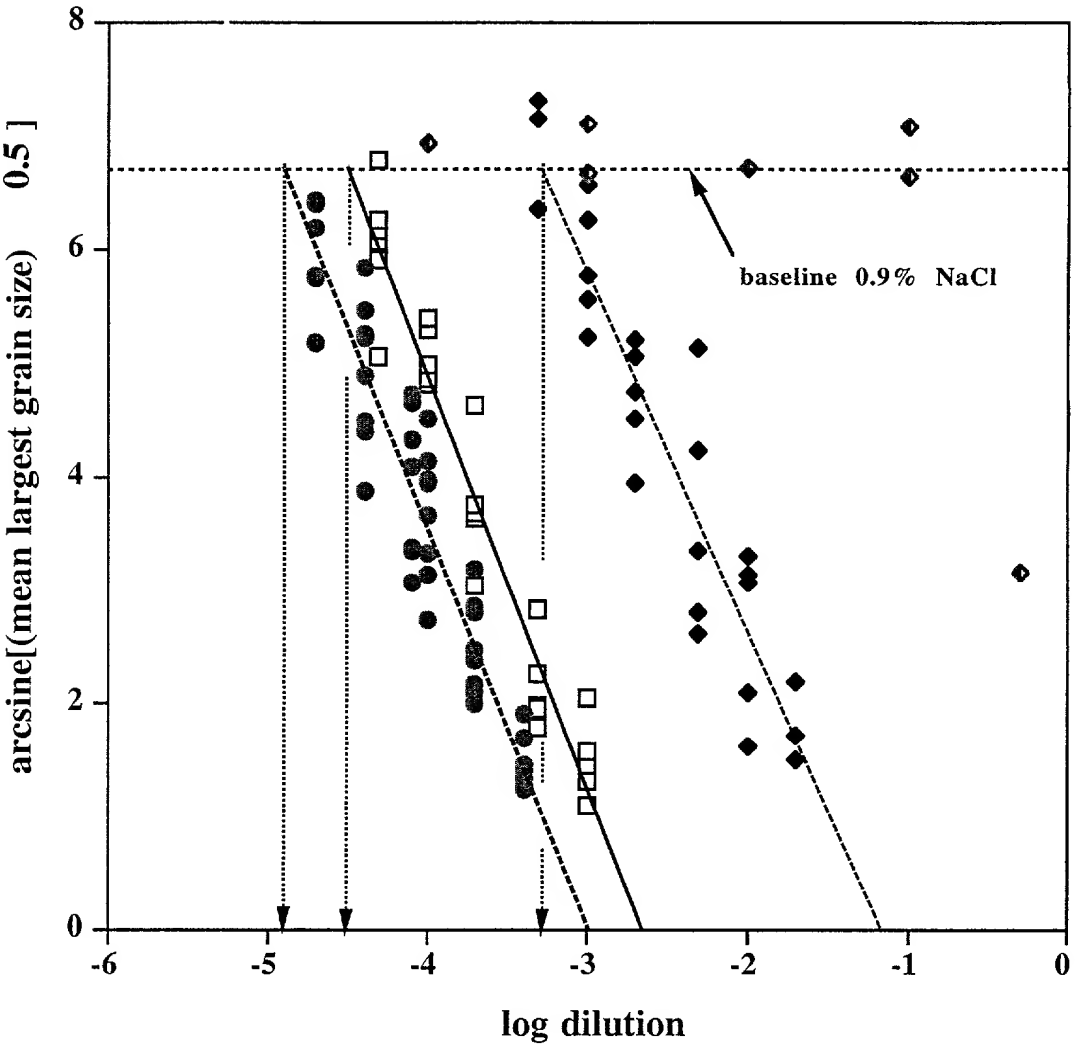

8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
arcsine[(mean largest grain size) 0.5]
log dilution
baseline 0.9% NaCl

FIG. 8.25

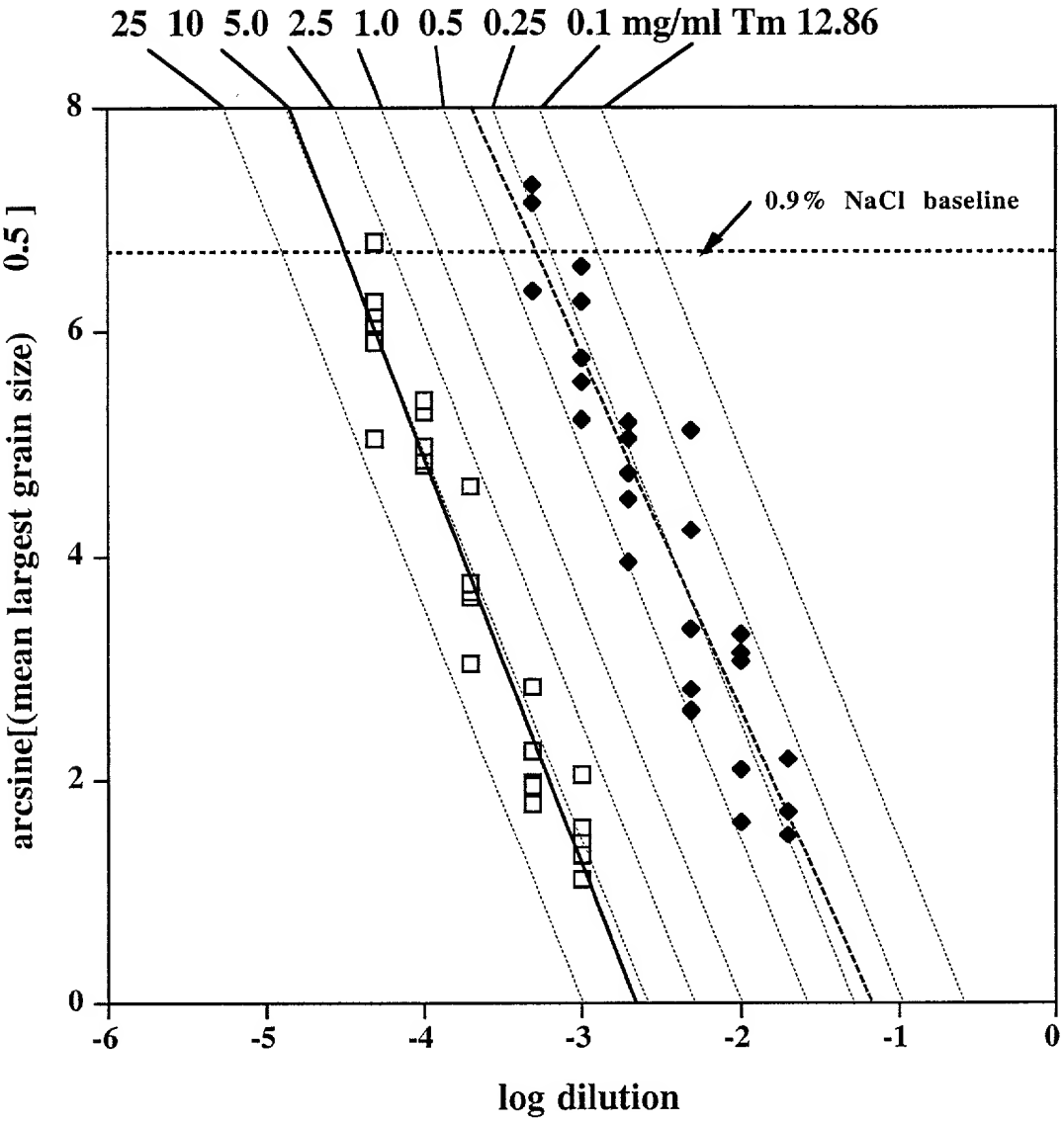

25 10 5.0 2.5 1.0 0.5 0.25 0.1 mg/ml Tm 12.86
0.9% NaCl baseline
arcsine[(mean largest grain size) 0.5]
log dilution
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0

FIG. 8.26

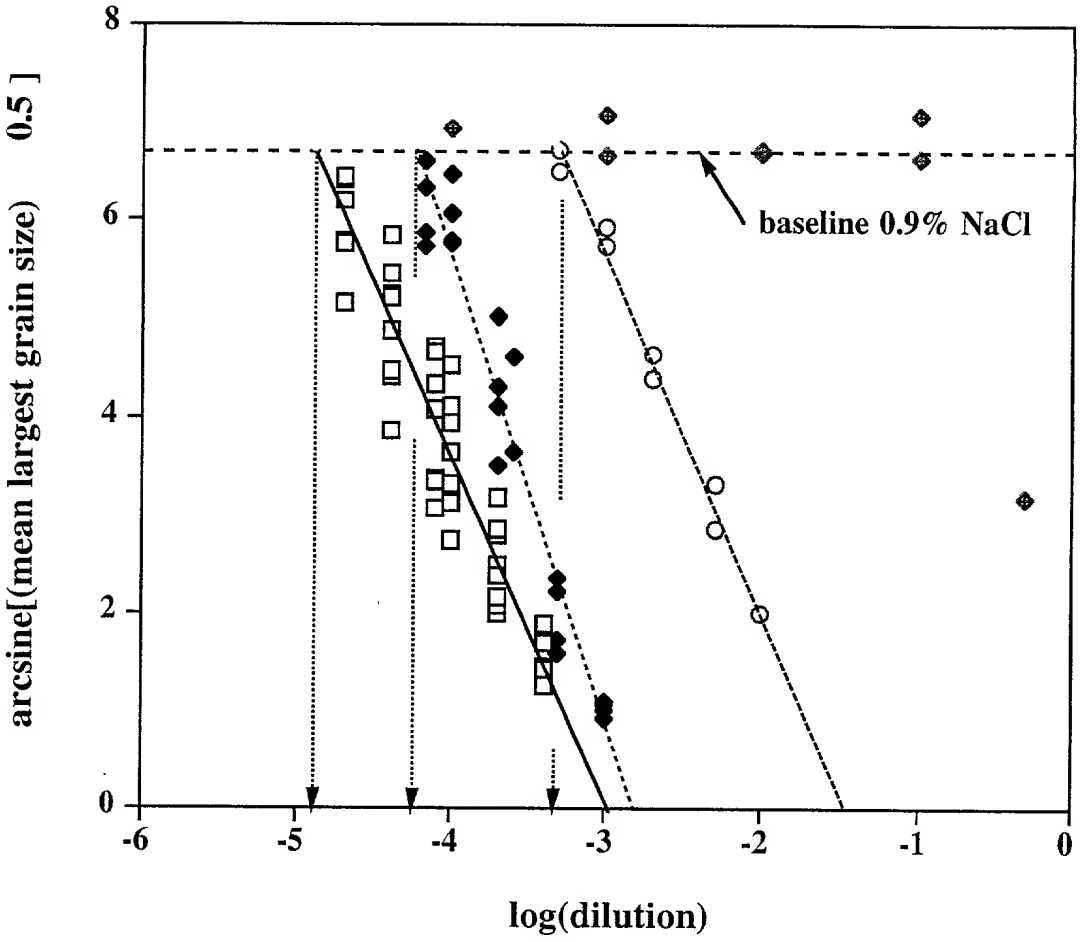

arcsine[(mean largest grain size) 0.5]
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
log(dilution)
baseline 0.9% NaCl

FIG. 8.27

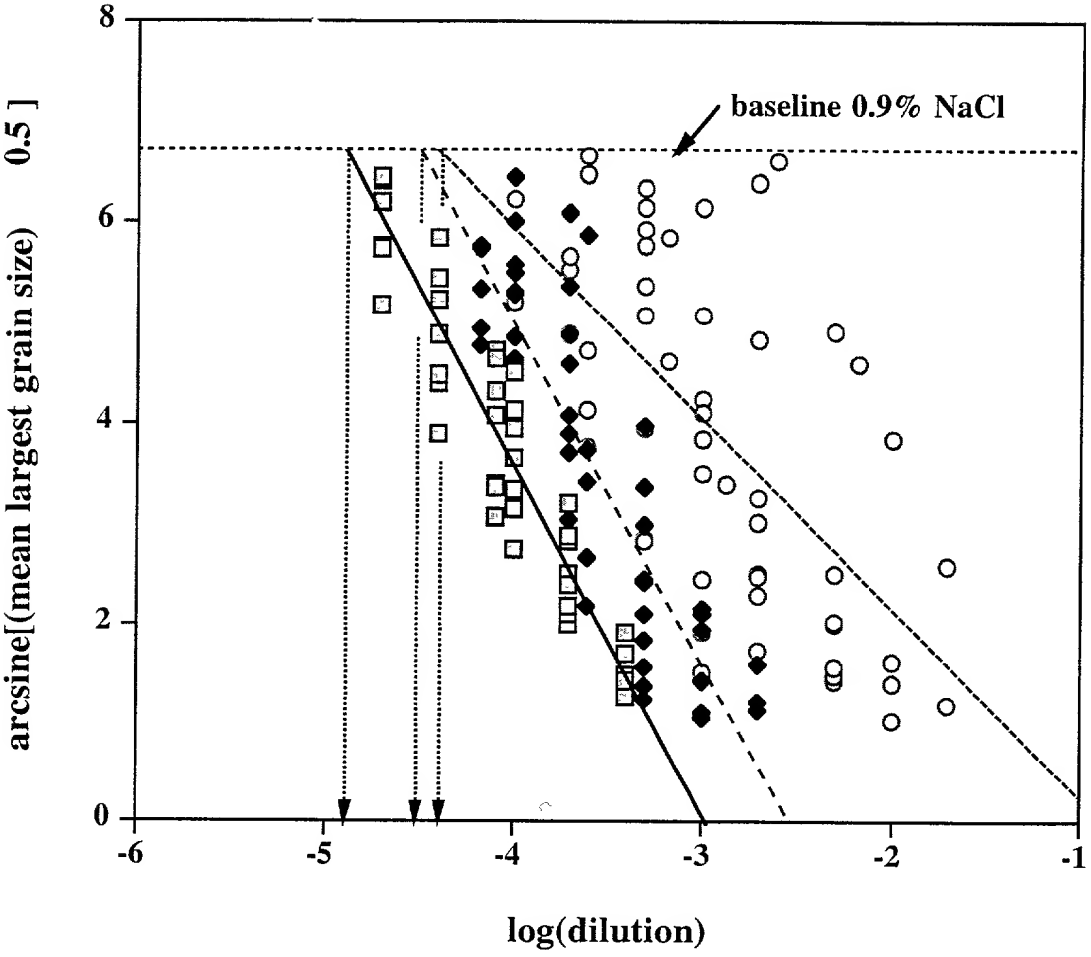

8
6
4
2
0
-6
-5
-4
-3
-2
-1
baseline 0.9% NaCl
arcsine[(mean largest grain size) 0.5]
log(dilution)

FIG. 8.28

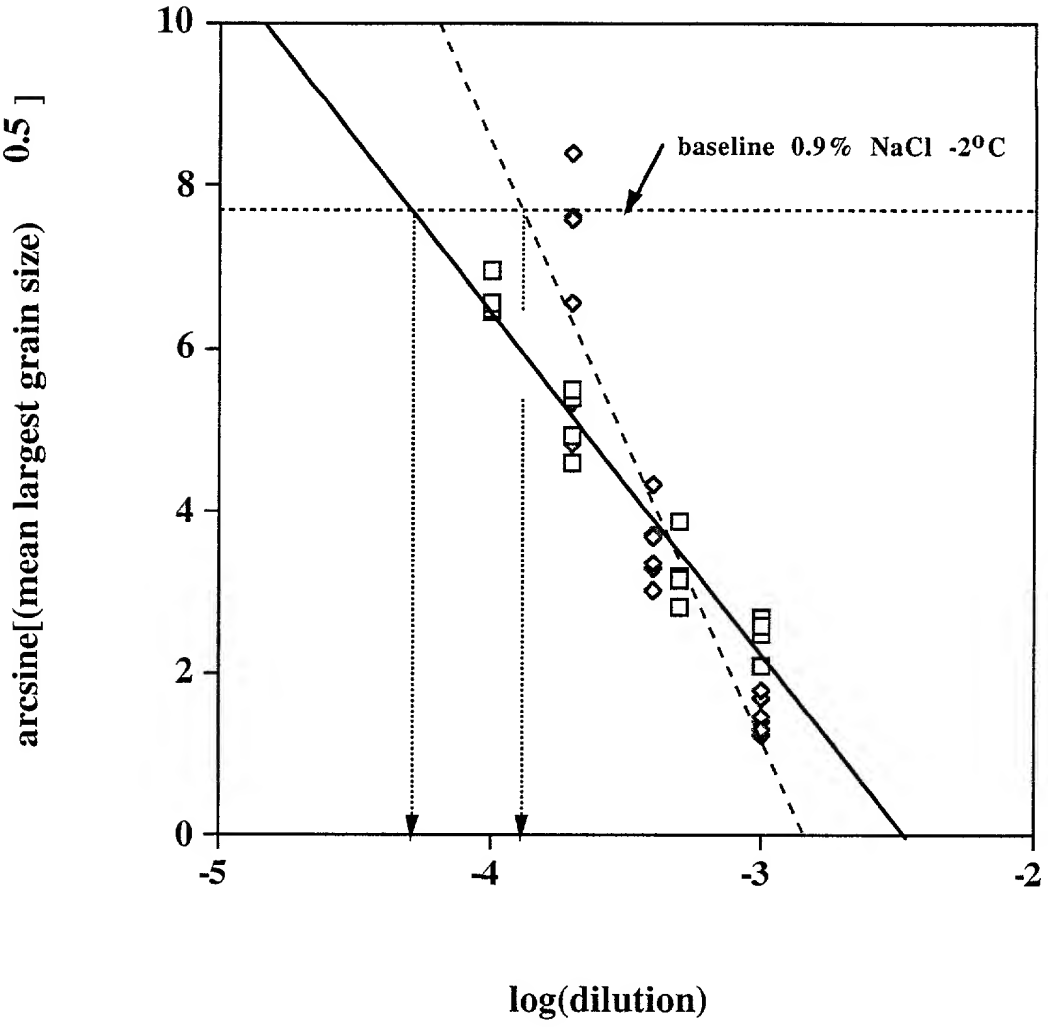

arcsine[(mean largest grain size) 0.5]
10
8
6
4
2
0
-5
-4
-3
-2
baseline 0.9% NaCl -2°C
log(dilution)

FIG. 8.29

EP 0 490 901 B1

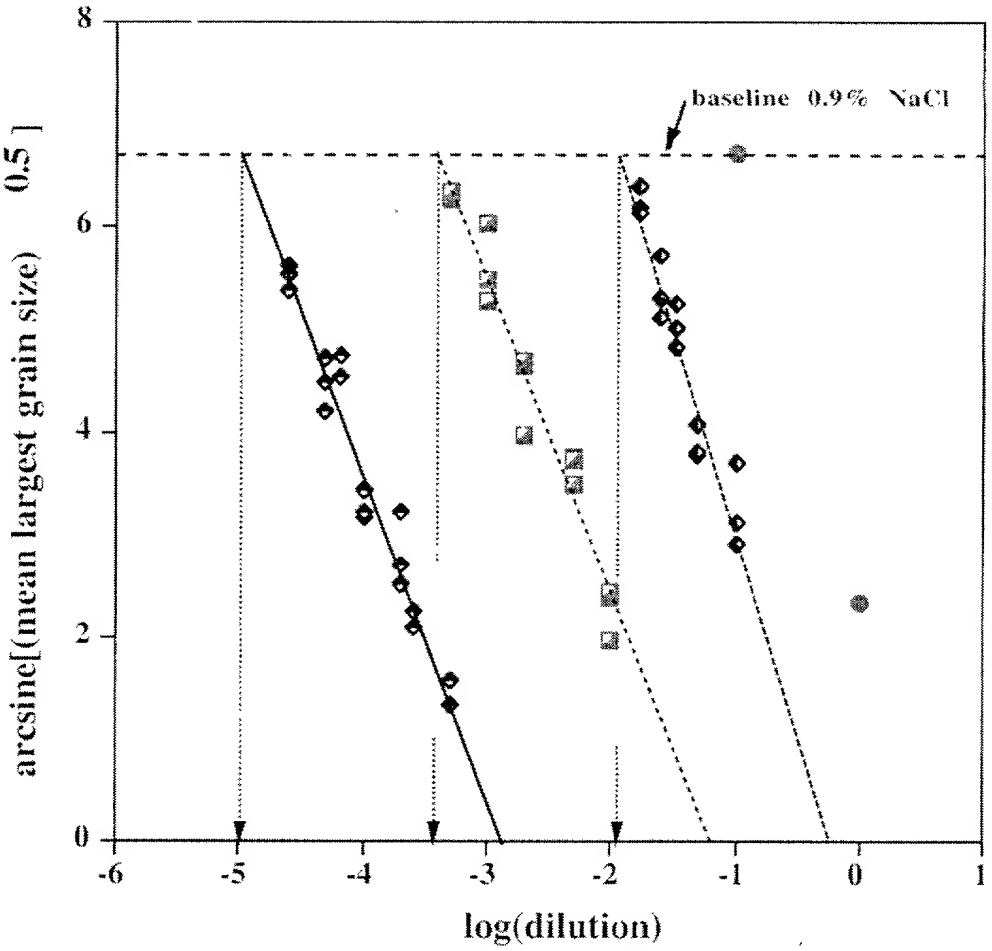


FIG. 8.30

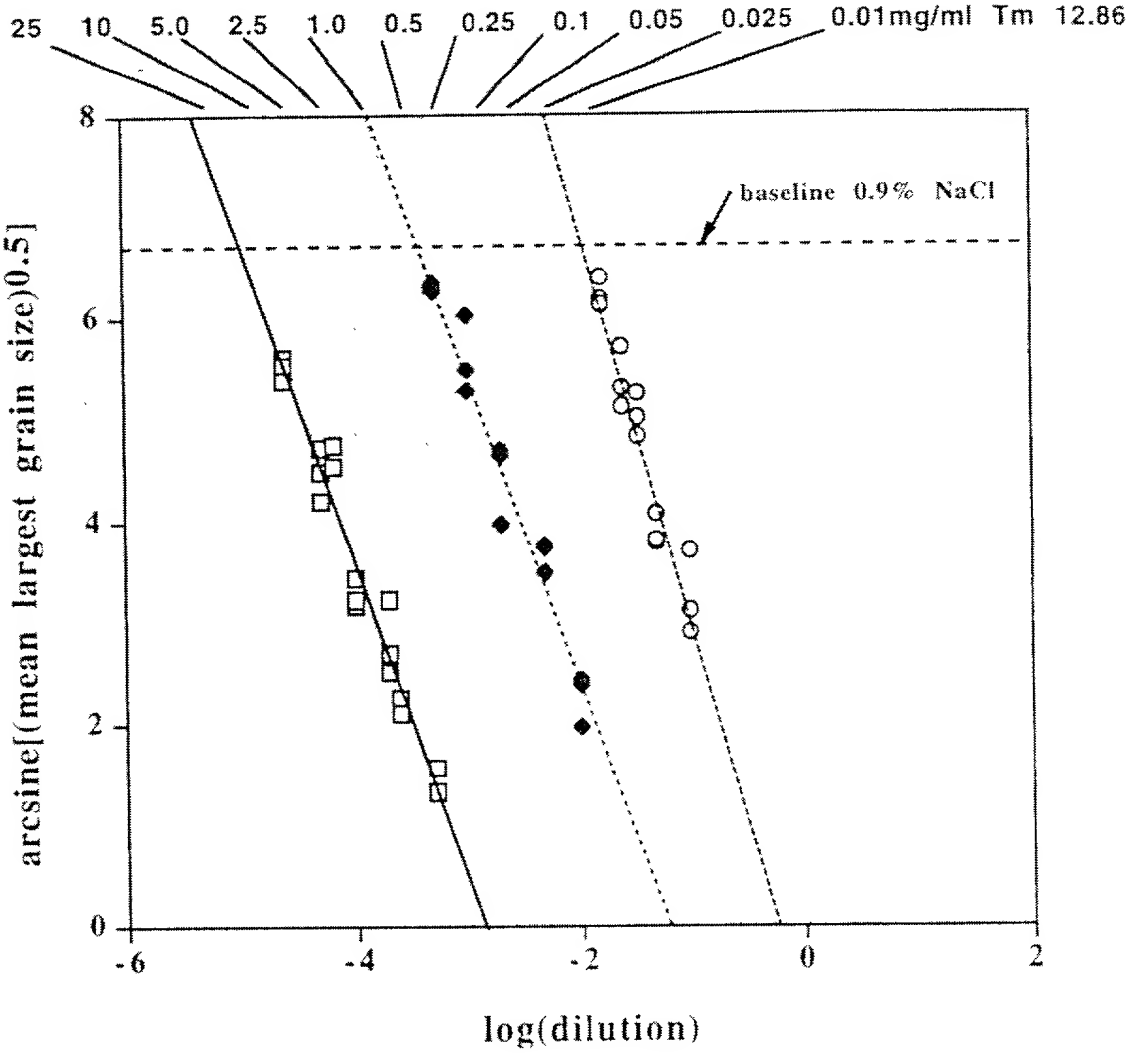

25
10
5.0
2.5
1.0
0.5
0.25
0.1
0.05
0.025
0.01mg/ml Tm 12.86
baseline 0.9% NaCl
arcsine[(mean largest grain size)0.5]
log(dilution)
8
6
4
2
0
-6
-4
-2
0
2

FIG. 8.31

EP 2 750 560 B1

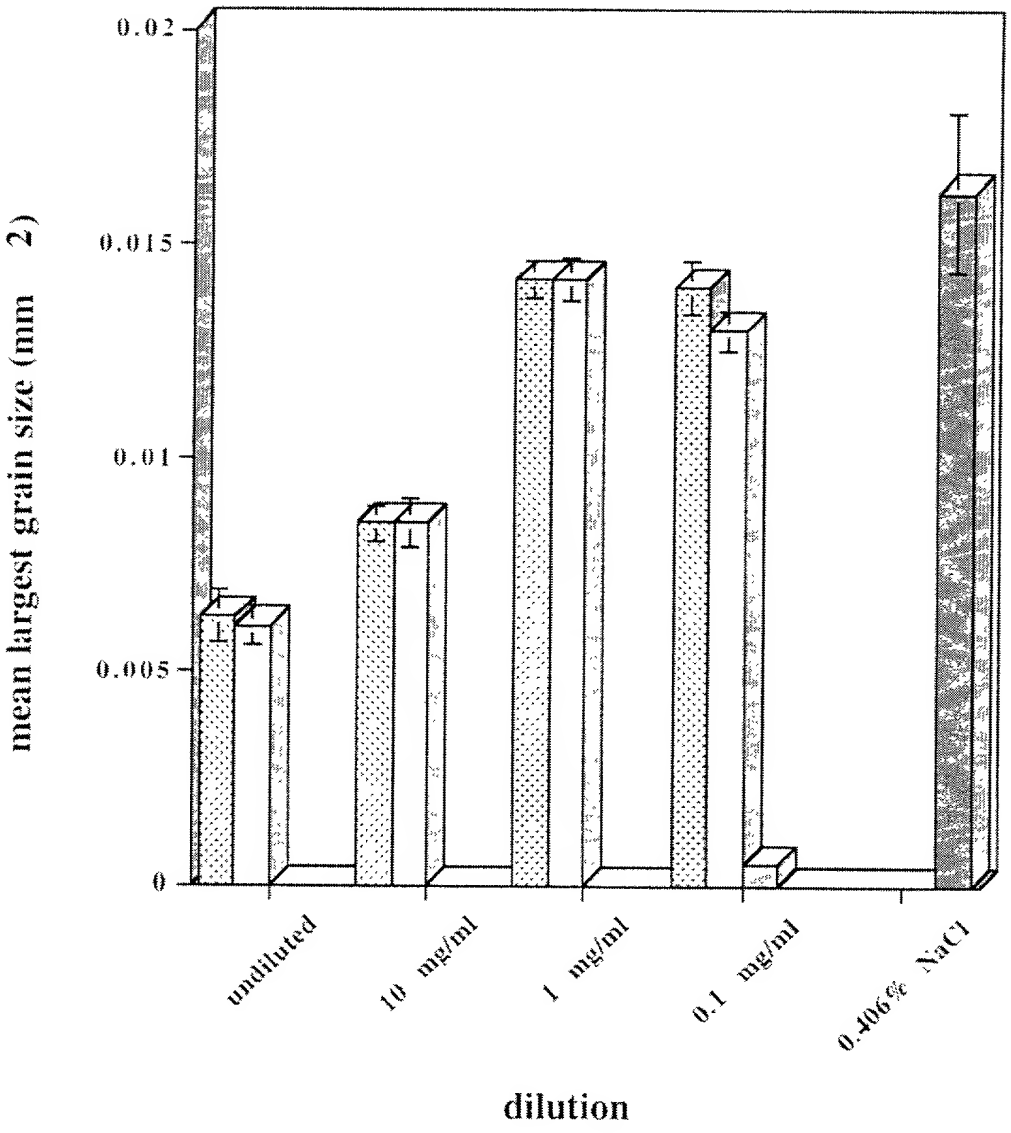


FIG. 8.32

EP 0 590 096 / 96

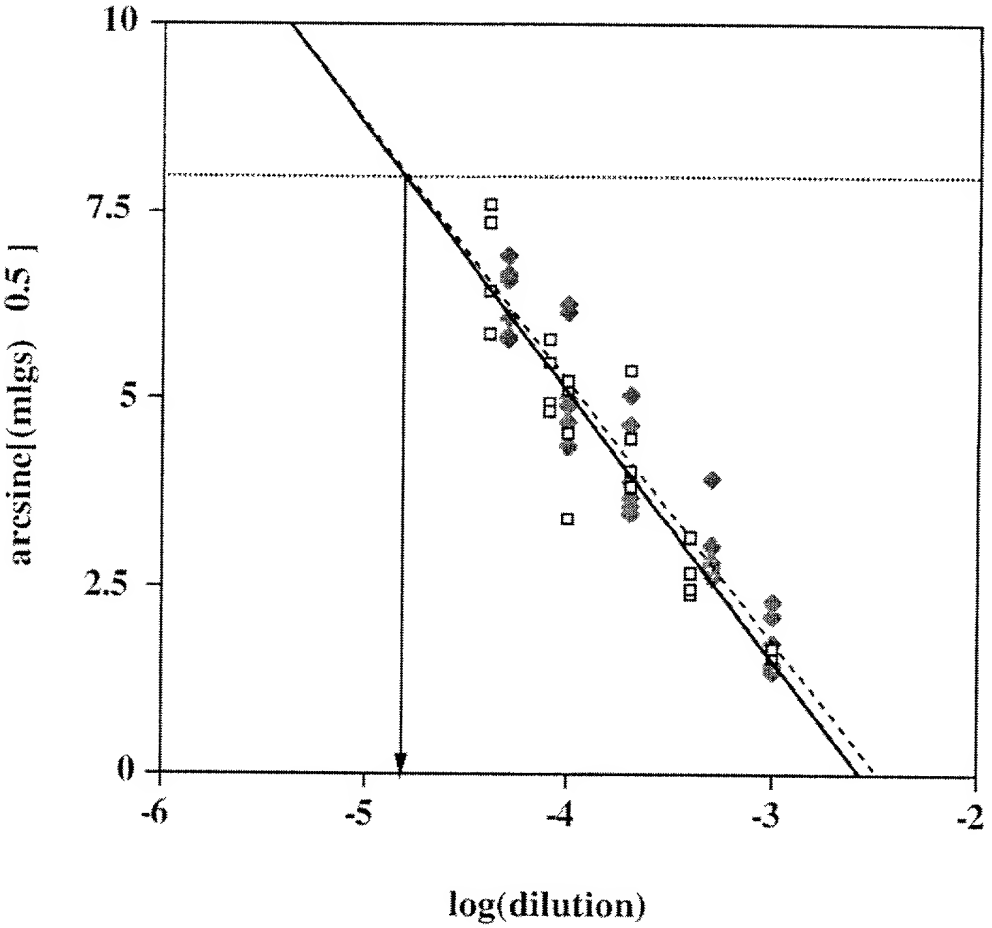


FIG. 8.33

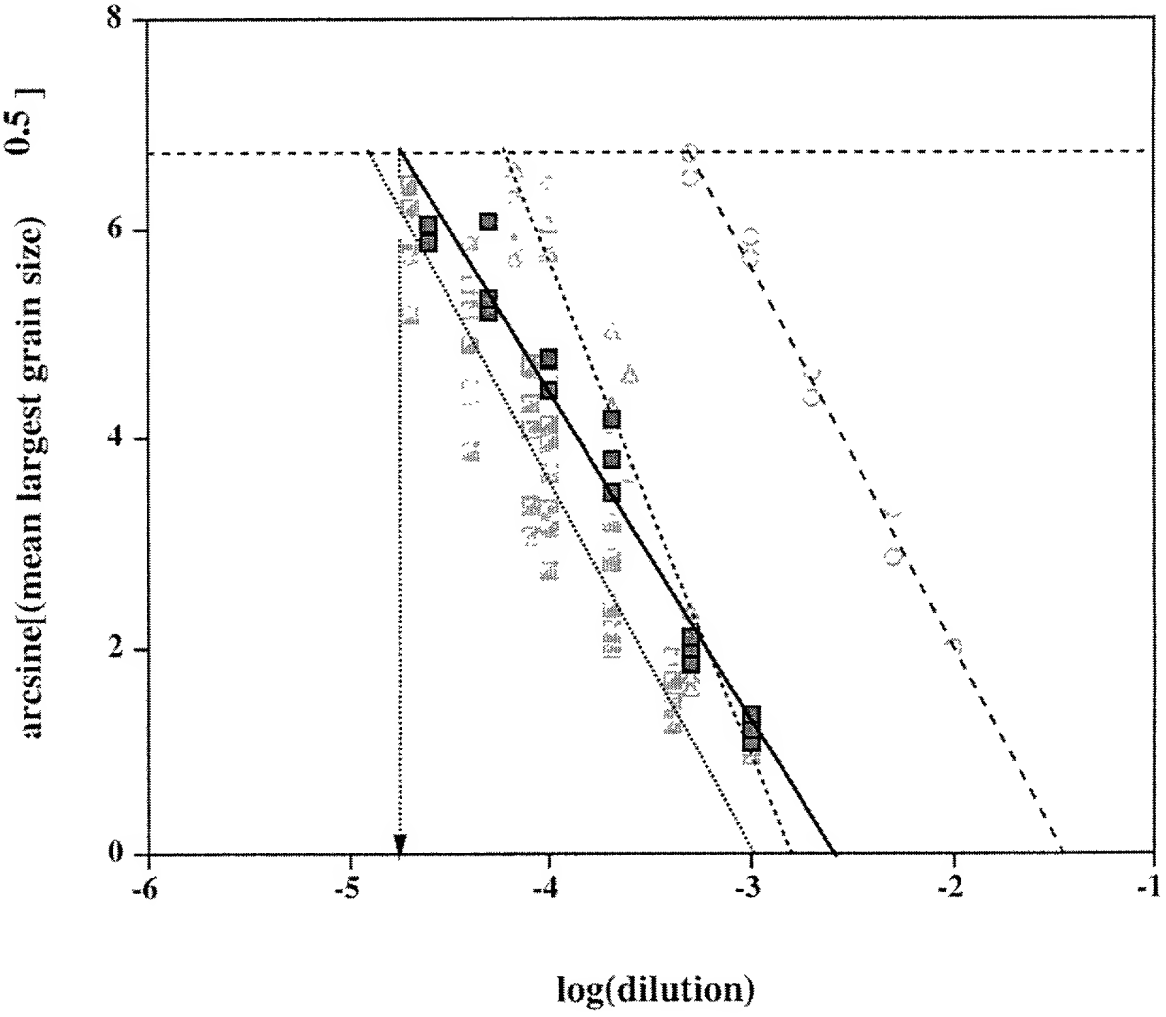

8
6
4
2
0
-6
-5
-4
-3
-2
-1
arcsine[(mean largest grain size) 0.5]
log(dilution)

FIG. 8.34

EP 2 969 991 B1 2019-06-26

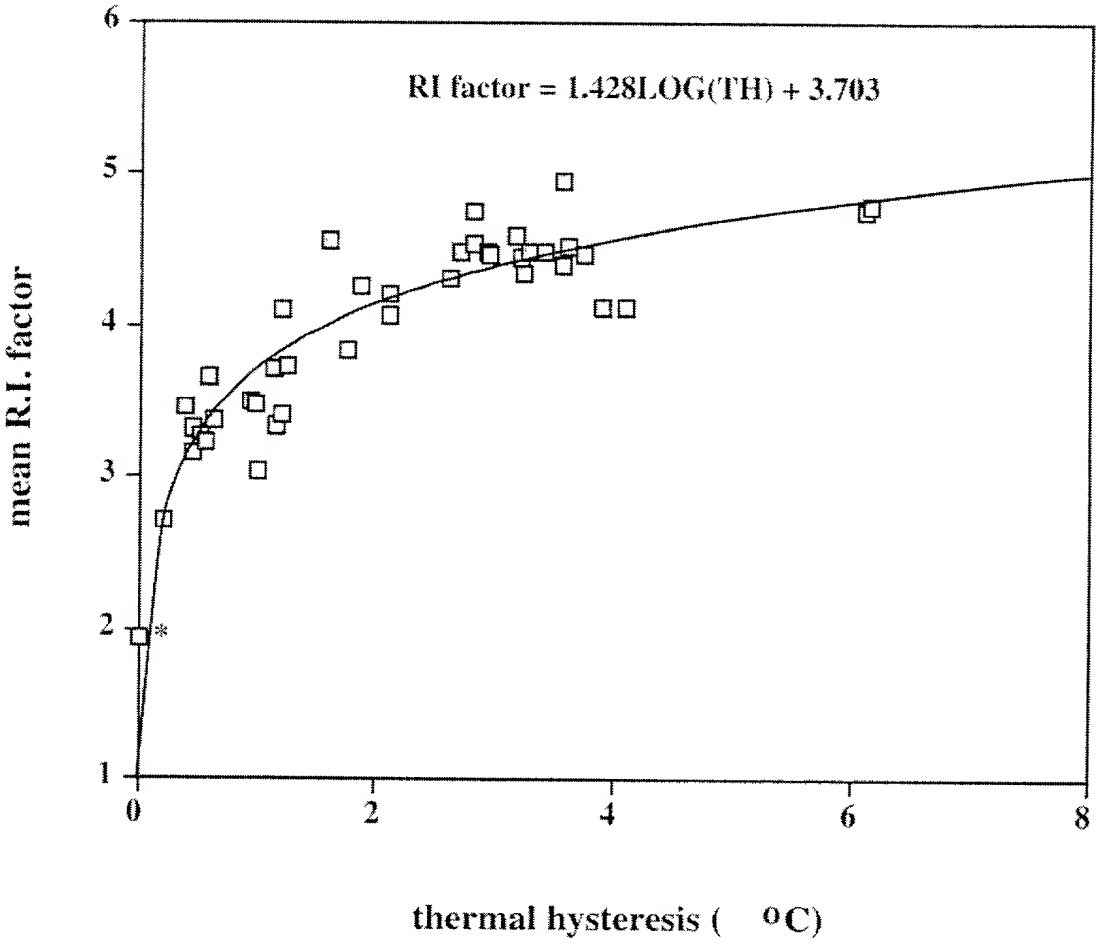


FIG. 8.35

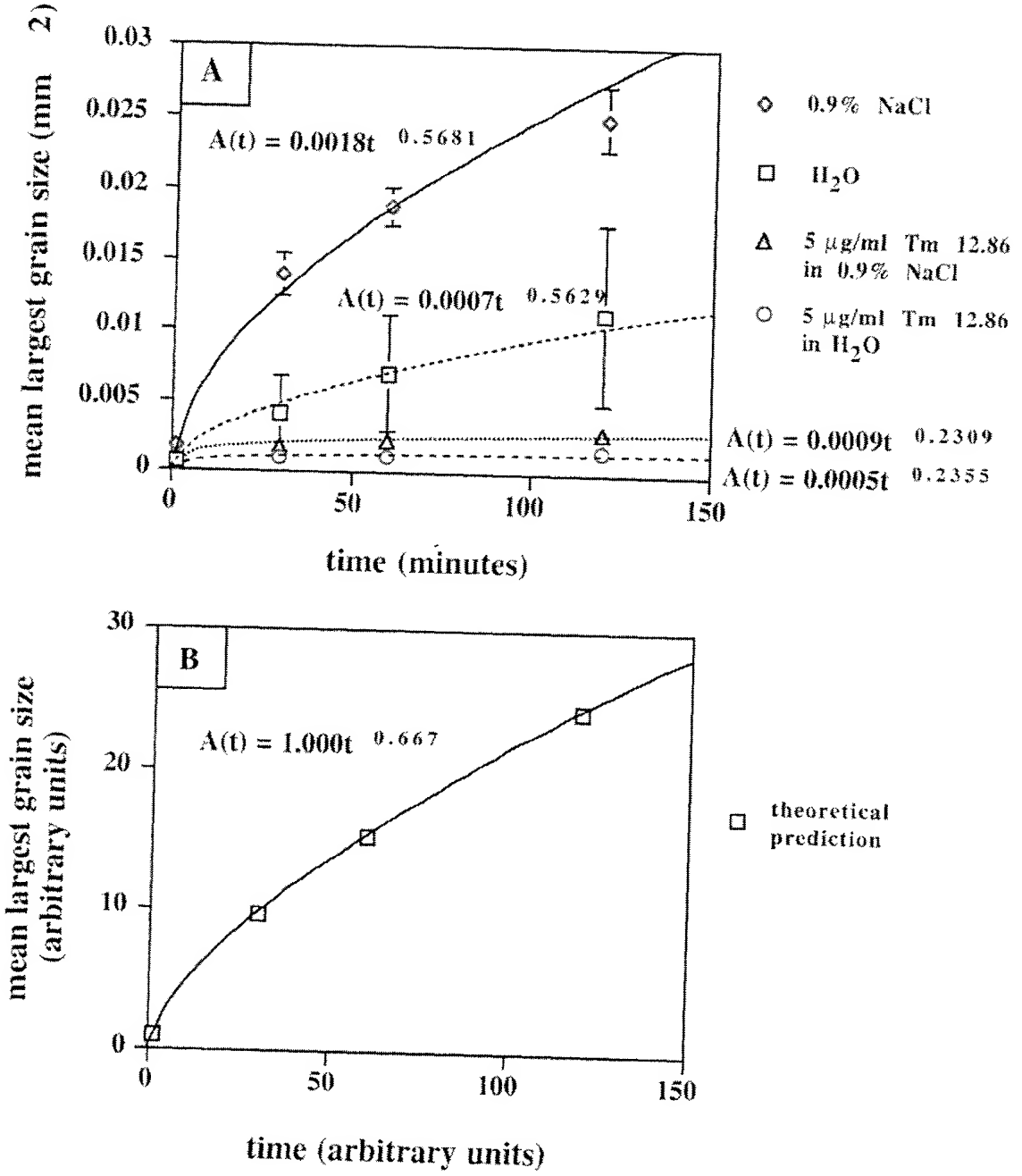

A
mean largest grain size (mm 2)
0.03
0.025
0.02
0.015
0.01
0.005
0
A(t) = 0.0018t 0.5681
A(t) = 0.0007t 0.5629
A(t) = 0.0009t 0.2309
A(t) = 0.0005t 0.2355
0.9% NaCl
H2O
5 µg/ml Tm 12.86 in 0.9% NaCl
5 µg/ml Tm 12.86 in H2O
0
50
100
150
time (minutes)
B
mean largest grain size (arbitrary units)
30
20
10
0
A(t) = 1.000t 0.667
theoretical prediction
0
50
100
150
time (arbitrary units)

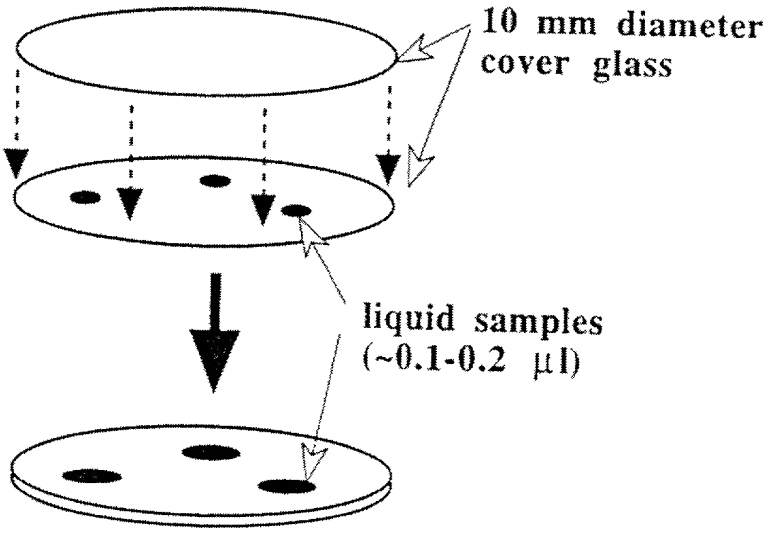
FIG. 8.36

A
log(mean largest grain size)
-1.5
-2
-2.5
-3
-3.5
0
0.5
1
1.5
2
2.5
log(time)
0.9% NaCl
H_2O
5 µg/ml Tm 12.86 in 0.9% NaCl
5 µg/ml Tm 12.86 in H_2O
B
log(mean largest grain size)
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
log(time)
theoretical prediction

FIG. 8.37

"Sandwich" method of R.I. assessment

1.



2.

3. FREEZE ON ~-80 C
   ALUMINUM PLATE (~10 MIN.)

4. PLACE ON COLD STAGE,
   ANNEAL AT -6 C UP TO
   12+ HOURS

FIG. 8.38

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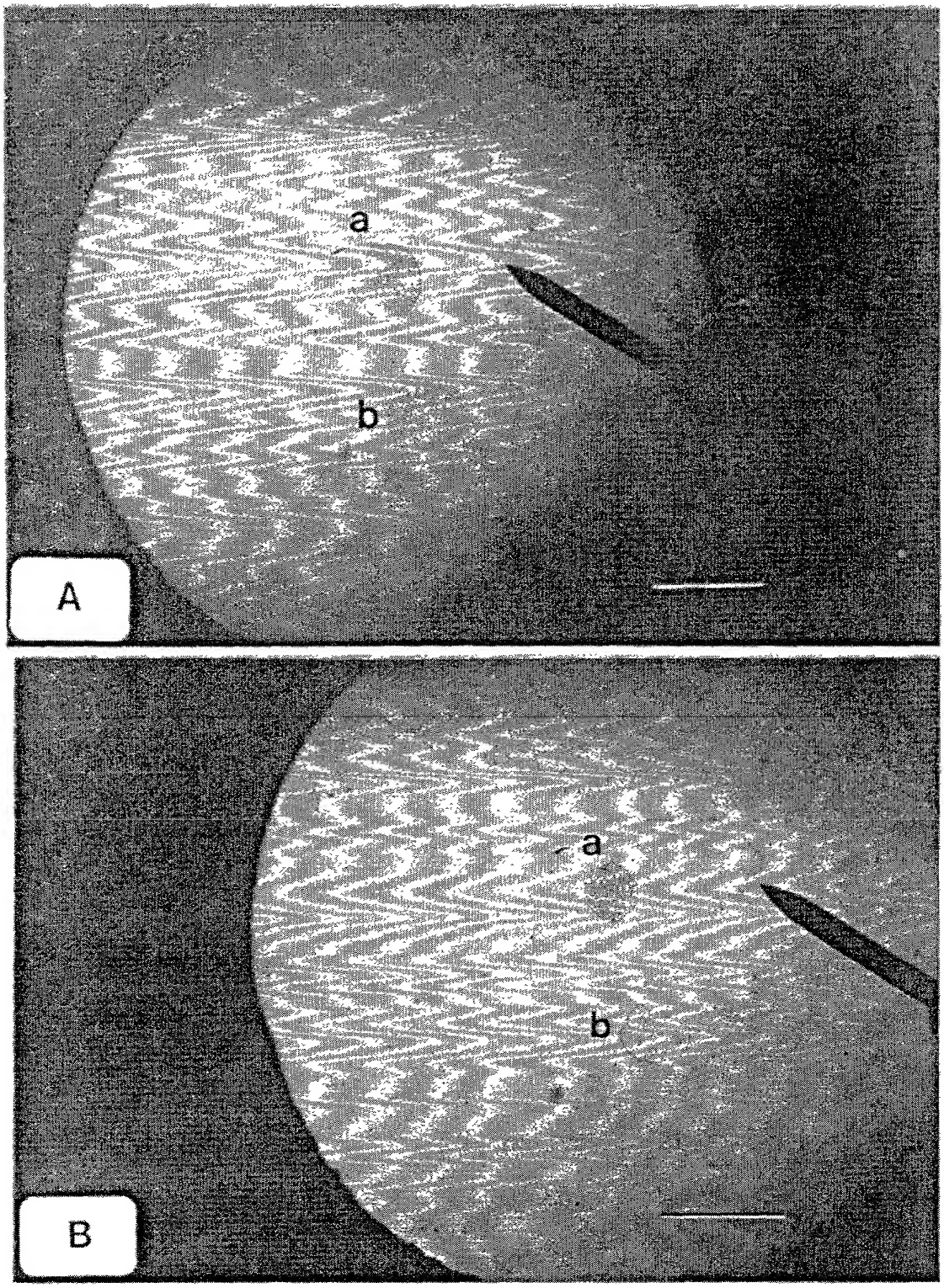

FIG. 8.39

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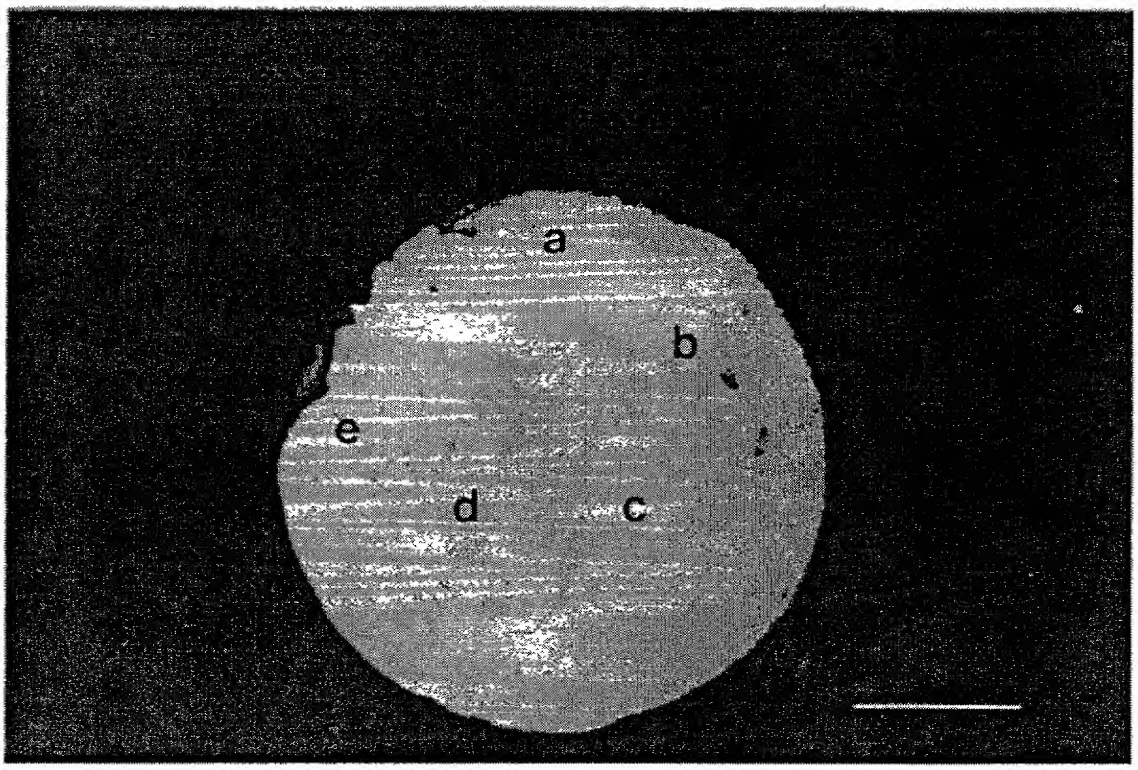

FIG. 8.40

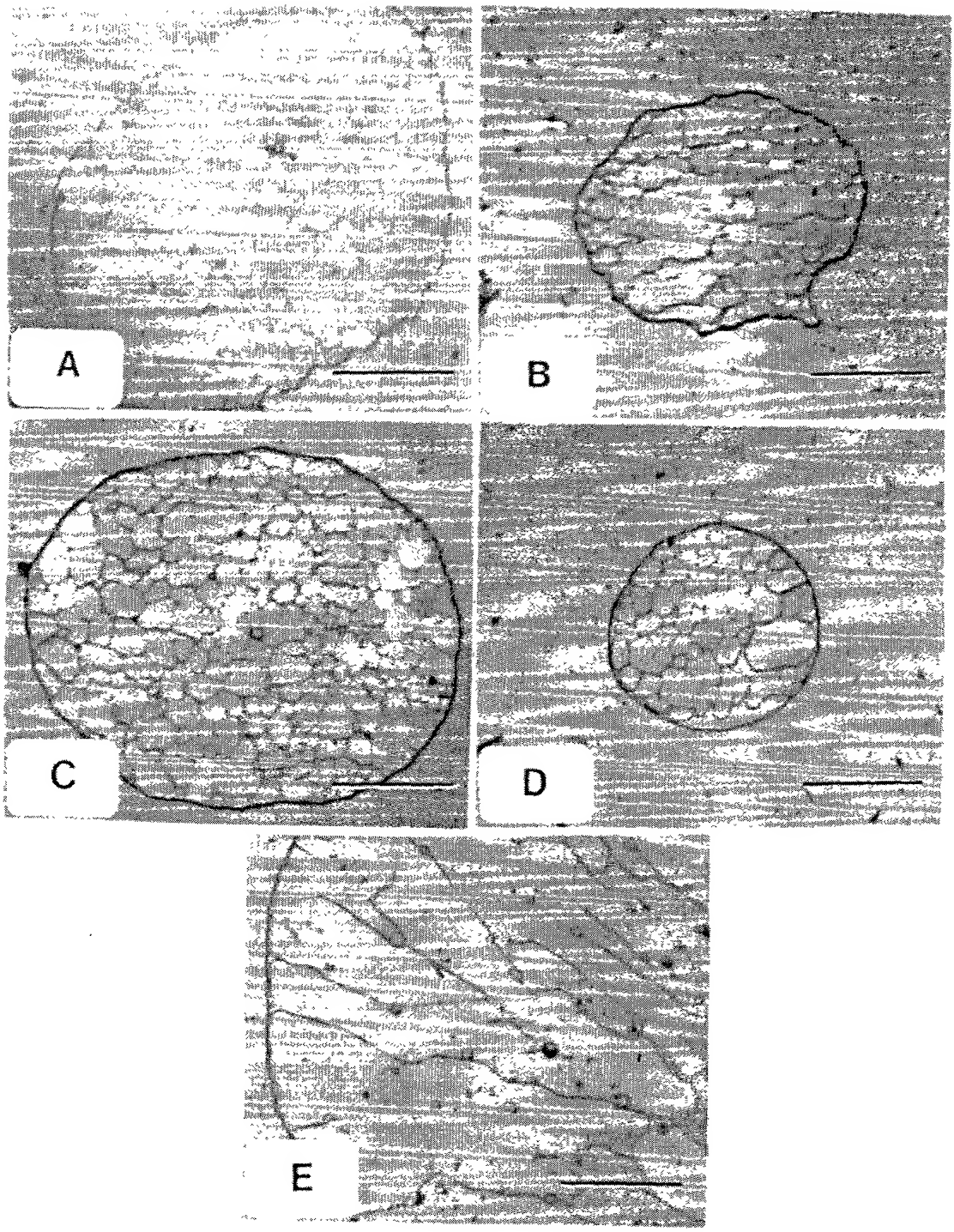

FIG. 8.41

Patent Application Publication

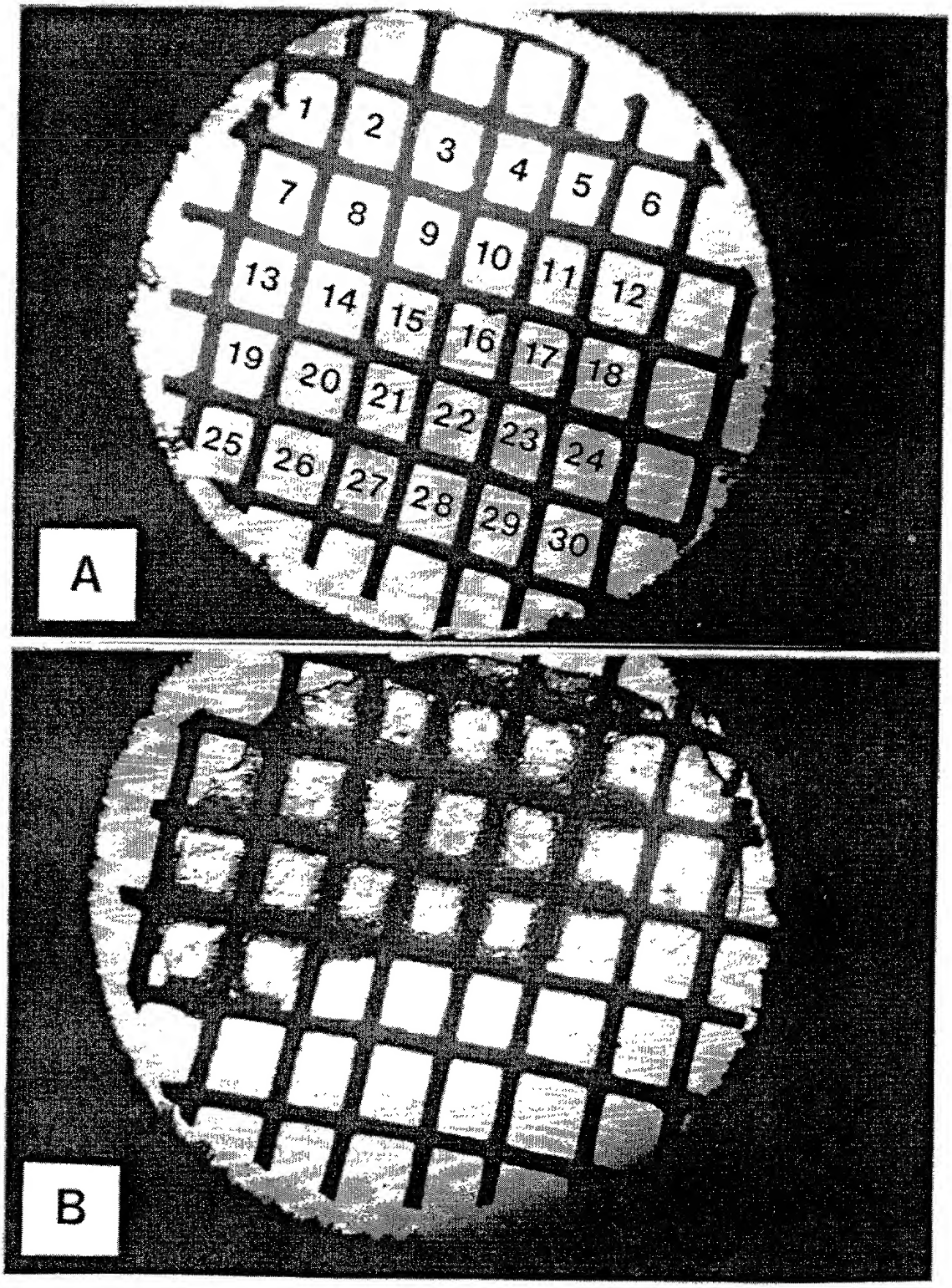

FIG. 8.42

DNA sequence of Tm 13.17 cDNA clone

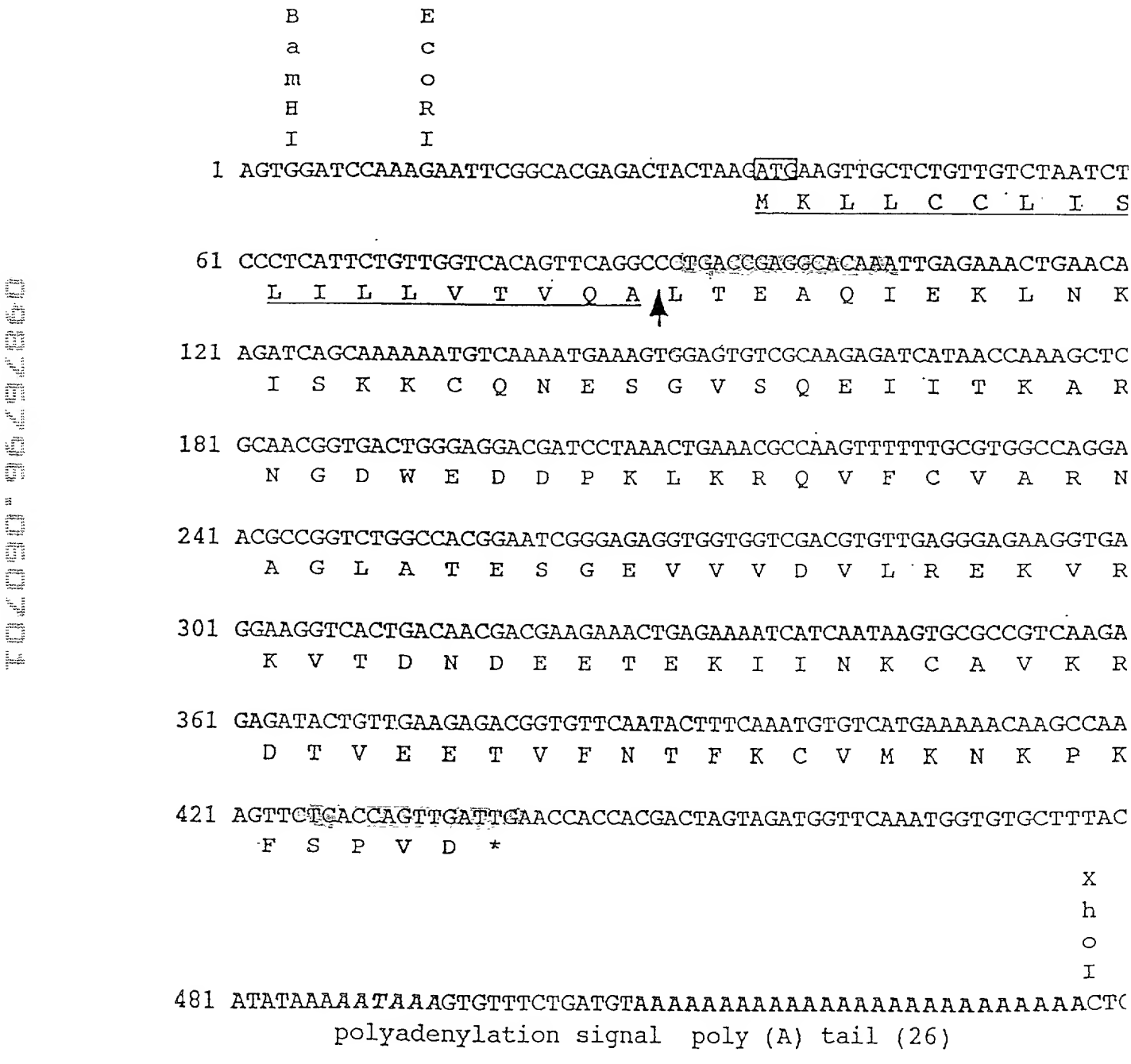

FIG. 8.43

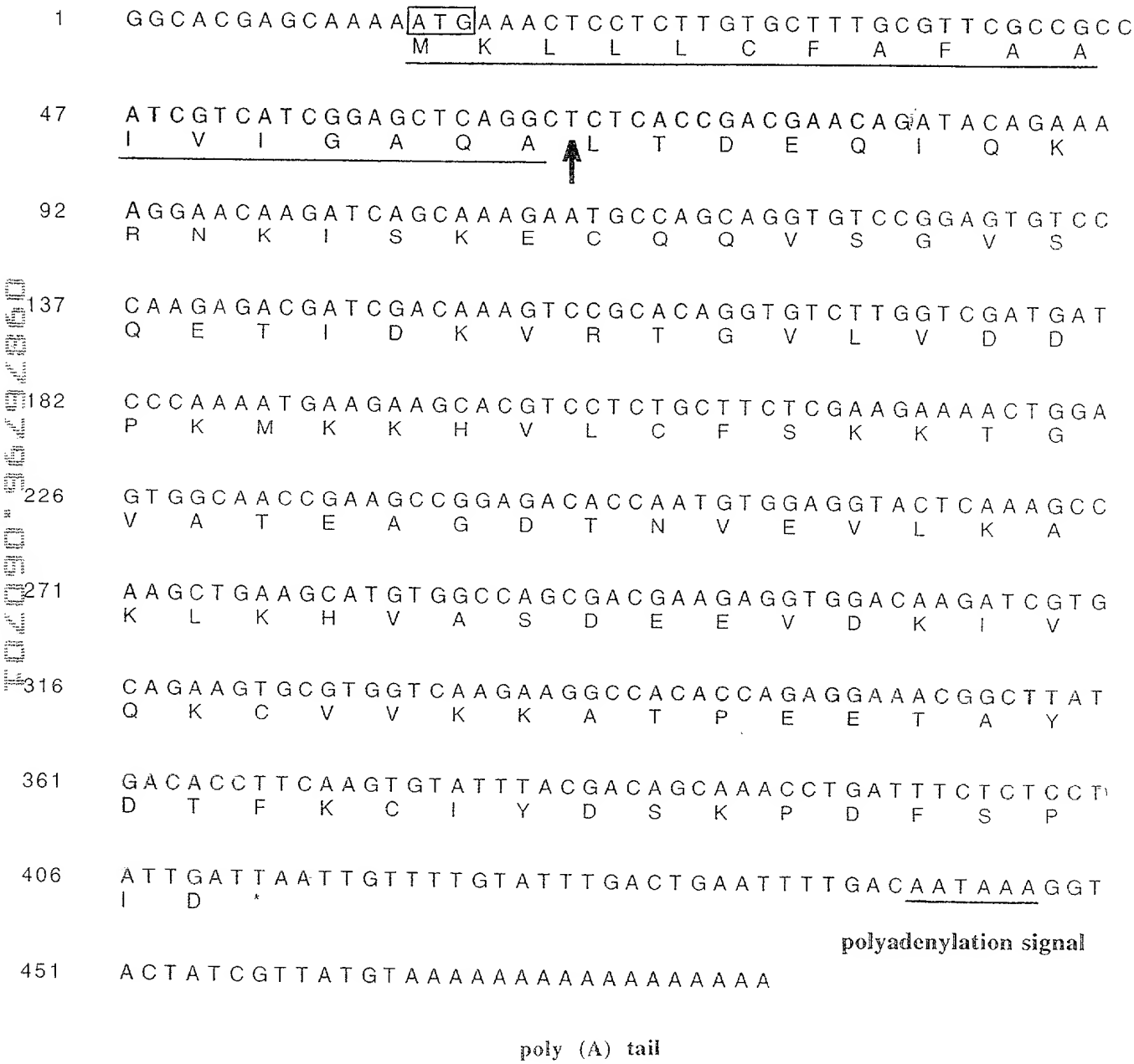
1 GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCCGCC
M K L L L C F A F A A
47 ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
I V I G A Q A L T D E Q I Q K
92 AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCC
R N K I S K E C Q Q V S G V S
137 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGATGAT
Q E T I D K V R T G V L V D D
182 CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
P K M K K H V L C F S K K T G
226 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
V A T E A G D T N V E V L K A
271 AAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTG
K L K H V A S D E E V D K I V
316 CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
Q K C V V K K A T P E E T A Y
361 GACACCTTCAAGTGTATTTACGACAGCAAACCTGATTTCTCTCCT
D T F K C I Y D S K P D F S P
406 ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
I D *
polyadenylation signal
451 ACTATCGTTATGTAAAAAAAAAAAAAAAAAA
poly (A) tail

FIG. 8.44